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A Thesis submitted

by

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Title

Investigations on the Constitution of the Polysaccharide of *Gigartina stellata*.

C O N T E N T S.

INTRODUCTION.

Although research into the chemistry of the polysaccharides of marine algae is only in its infancy, it is already apparent that the group, known as ethereal sulphates, will play an important part in the chemistry of these polysaccharides, as well as in their industrial application. Marine algae have been divided into four main groups (1), depending on the pigment which predominates:-

Blue-green. (Myxophyceae).	Green. (Chlorophyceae).	Brown. (Phaeophyceae).	Red. (Rhodophyceae).

The brown sea-weeds are important in that they contain mannitol, alginic acid, laminarin, and a low-grade cellulose, in addition to the ethereal sulphate, fucoidin.

The red sea-weeds, on the other hand, apparently do not contain alginic acid, but a number of them have been found to give ethereal sulphates on extraction with water or dilute acid. Since less than half a dozen ethereal sulphates have so far been examined, it is obvious that any attempt to classify this group of compounds would be premature. It is interesting to note, nevertheless, that some of these have already found an application in industry. The most important polysaccharide yet obtained from the red sea-weeds is agar, the gel of which is extensively used for bacteriological culture purposes. It is extracted by hot water/

water from various species of Gelidium, but the sulphur content of most samples of agar is so low that it is not generally classed as an ethereal sulphate (2).

Gigartina stellata Batt., formerly known as Gigartina mamillosa J.G. Agardh, is a red sea-weed widely distributed around our shores. It is similar in form to Chondrus crispus, and is collected with that species and marketed under the name of Carragheen or Irish moss. Carragheen was used as a thickener of jams and jellies, as a size, and as an emulsifying agent in pharmacy. But since World War II, when the supply of Japanese agar was cut off, Carragheen has been used in the preparation of 'British agar', which is said to be highly satisfactory for bacteriological work. Extensive research on 'British agar' is at present in progress with a view to its further development, and the harvesting of these sea-weeds, along with other closely related species, is restricted by a Government order (3).

Before outlining briefly the chemistry of the ethereal sulphates, it seems advisable to summarise, as far as possible, the characteristics of this group of compounds:-

Polysaccharide ethereal sulphates are extracted from the sea-weeds, with water or dilute acid, as salts, and purified by prolonged dialysis. The simplest general/

general formula for a polysaccharide ethereal sulphate is:-

$$(R.O.SO_2.OM)n$$

$R.O.SO_2.OM$ represents the repeating unit in the polysaccharide.

R = the monosaccharide repeating unit.

M = Na, K, $\frac{Ca}{2}$, or $\frac{Mg}{2}$.

n is unknown in most cases.

In general, R is not a simple monosaccharide, but a mixture of two or more carbohydrate residues.

In a compound of this formula the sulphate will not be ionised, and an aqueous extract of an ethereal sulphate gives no precipitate with barium chloride, until after hydrolysis with hydrochloric acid. The metal, on the other hand, will be ionised, so that in the case of a calcium polysaccharide ethereal sulphate, for example, the calcium can be quantitatively precipitated with ammonium oxalate.

On ignition an ethereal sulphate gives an ash, consisting of M_2SO_4, and it is evident that during ignition half the sulphate is lost as sulphur trioxide:-

$$2R.O.SO_2.OM = M_2SO_4 + SO_3 + \text{products of combustion.}$$

On hydrolysis with acids an ethereal sulphate gives the free sugar, or sugars, together with sulphuric acid:-

$$R.O.SO_2.OM + HCl + H_2O = R.OH + H_2SO_4 + MCl$$

Hence the sulphate content of the ash will only be half that/

that contained in the hydrolysis mixture. This 1:2 ratio of sulphate in the ash to sulphate after hydrolysis is characteristic of an ethereal sulphate.

In practice it is found that this 1:2 ratio does not always hold, and the sulphate in the ash is often very much less than half the sulphate after hydrolysis. In some cases, this may be due to the fact that the ethereal sulphate cannot be represented by such a simple formula, but in others the difference can be explained by the loss of sulphur from the ash. During the process of incinerating the ethereal sulphate, the large amount of carbon present may reduce the sulphate to sulphite, or even to sulphide. Hence, during ignition, sulphur may be lost from M_2SO_3 as SO_2, while in dissolving the ash in hydrochloric acid sulphur may again be lost from M_2S as H_2S. This difficulty has been overcome by converting the ash to sulphate with sulphuric acid before estimating sulphate. Certainly, in the present study, concordant results could only be obtained by determining the ash as sulphate. Nelson and Cretcher (4), and Lunde, Heen and Øy (5) have also referred to this low sulphate in the ash unless the ash is determined as sulphate.

It is becoming increasingly evident, as research in this group of compounds advances, that these polysaccharides are to be characterised more by the constituent/

constituent sugars present than by the metallic ions attached to the ethereal sulphate. The same ethereal sulphate may be obtained as a calcium salt from one alga and as an alkali metal salt from another. Two different salts of the same ethereal sulphate may even be isolated from the same alga, if the two samples of alga are collected at different parts of the world, or if the method of extraction is varied. For example, the alkali metal salt may be soluble in cold water while the calcium salt may be insoluble, in which case the cold extract will contain chiefly the alkali metal salts and the hot extract will be chiefly the calcium salt.

The water-soluble polysaccharide ethereal sulphate, fucoidin, occurs along with the water-insoluble poly-uronide, alginic acid, in various, common, brown sea-weeds. Fucoidin was first named and isolated by Kylin (6) from various species of Laminaria and Fucus, and later examined by Bird and Haas (7), and Lunde, Heen and Öy (5).

A corresponding water-soluble carbohydrate was isolated from Macrocystis pyrifera by Hoagland and Lieb (8), and this was shown to contain an ethereal sulphate grouping by Nelson and Cretcher (4).

Bird and Haas (7) isolated fucoidin by soaking Laminaria spp. in distilled water, purifying the extract/

extract by dialysis, and precipitating in alcohol. The pure product had an ash content of 30.93%, which was found to be chiefly calcium sulphate. Sulphate in the ash (15.10%) was shown to be half the total sulphate (30.33%), which suggested fucoidin as the calcium salt of a polysaccharide ethereal sulphate. This view was strengthened by the evidence that the calcium was ionised, while the aqueous extract gave no reaction for sulphate ion.

Lunde, Heen and Öy (5) recognised that the purification of fucoidin was a matter of great difficulty, and, in order to obtain a standard preparation, started, not by extracting the whole sea-weed, but by collecting the colourless, viscous drops, which are exuded from Laminaria digitata on standing in air; their samples were collected on the south-west coast of Norway. The ash content was shown to be 26-30%, while analysis of the ash indicated that it, unlike the ash of the Bird and Haas product, consisted chiefly of sodium sulphate with some potassium, calcium and a small quantity of magnesium sulphate. Sulphate in the ash (17-19%) was approximately half the total sulphate (35.5-37.5%), thereby confirming the presence of an ethereal sulphate grouping as shown by Bird and Haas (7).

Of the carbohydrate portion of fucoidin very little is known. Kylin (6) claimed the presence of pentoses and/

and methylpentoses, and the existence of the methylpentose, fucose, was confirmed by Bird and Haas (7) by the isolation of an osazone, m.p. 170 - 173°, from the products of hydrolysis. The preparation of fucose from Fucus spp. was first described by Gunther and Tollens (9), by Votoček (10) and by Clark (11), who hydrolysed the entire sea-weeds after a preliminary soaking in dilute acid. Bird and Haas (7) also obtained evidence of the presence of an uronic acid (7.3%), which was also found by Nelson and Cretcher (4) in the product from Macrocystis pyrifera. This, however, was not assumed to be a part of the fucoidin, but to come from the alginic acid, the main carbohydrate constituent.

Lunde, Heen and Øy (5) could find no evidence for the existence of pentose or uronic acid. They estimated the fucose content to be 33-37%, by distillation with 13% hydrochloric acid and weighing the methylfurfural formed as the phloroglucide. This corresponds to about 60% of the calculated quantity, on the assumption that the carbohydrate residue consists exclusively of fucose. According to Lunde, Heen and Øy (5), the sum of the components of fucoidin, i.e. metals, sulphate and fucose, make up about 80% of the substance, which still leaves about 20% to be identified. They have represented fucoidin by the formula:-

$$(R.R'\text{-}O.SO_2.O\text{-}M)_n$$

where/

where R' is an unknown carbohydrate.

Barry and Dillon (12) have recently described the isolation of a galactan sulphuric acid from Dilsea edulis, a red sea-weed closely allied to Gigartina stellata. The mucilage was extracted with dilute hydrochloric acid and precipitated in alcohol as a tough, fibrous solid. The ash content was reduced by repeatedly precipitating the solution in dilute hydrochloric acid from alcohol, and more effectively by dialysis, first against dilute sulphuric or hydrochloric acid, and then distilled water, to give a white, granular powder; ash, 6.4%; $[\alpha]_D$ + 47°. This was an acid, a 1% solution of which in water had p H 4, with an equivalent of 627. It was shown to be an ethereal sulphate by giving barium sulphate after boiling with hydrochloric acid and barium chloride. Barry and Dillon (12) experienced difficulty in determining total sulphate, due to the presence of small amounts of inorganic sulphate as impurity. Ultimately, their method was to add excess barium chloride to a solution of a weighed quantity, allow the solution to stand for 12 hours, filter off the barium sulphate, and boil the filtrate with dilute hydrochloric acid. (Total sulphate, 12.0%; calculated for the ash-free substance).

Hydrolysis of the galactan sulphuric acid with acid showed galactose to be present to the extent of about/

about 60%, determined as mucic acid. No other sugars could be isolated. The ester, moreover, was attacked by periodic acid, which is specific for α- glycol linkages, to give an oxidised product, which on hydrolysis gave glyoxalosazone and galactosazone. This suggests that some galactose units are oxidised, while others are not.

The total sulphate figure of 12.0% is much lower than that demanded by a pure galactan sulphuric acid, containing one sulphate group attached to every galactose unit, and corresponds only to one group for every 4-5 galactose units. It should be pointed out here that it may be a somewhat dangerous procedure to attempt to isolate polysaccharide sulphuric acids in the free state. These should be strong acids, $(R.O.SO_2.OH)_n$, which will tend to undergo autohydrolysis on concentrating the aqueous solution in vacuo, and, more particularly, on drying the solid above room temperature. It was found in the present study, for example, that an aqueous solution of the free acid obtained from Gigartina stellata became reducing on heating at 100° for 10 minutes, with the liberation of free sulphuric acid. This may possibly explain Barry and Dillon's difficulty in obtaining a sample of the galactan sulphuric acid completely free from sulphate ions.

From/

From the point of view of the present study the most important ethereal sulphates are those obtained from Chondrus crispus, which, as indicated earlier, is a dark-purple sea-weed, constituting, either alone or together with Gigartina stellata, the Carragheen or Irish moss of commerce.

Although the early work on Chondrus crispus is extremely complex and the results discordant, due chiefly to the fact that the early investigators worked on the sea-weed as such rather than on the aqueous extract, nevertheless the following points are worthy of mention. In 1868 Flückiger and Obermayer (13) reported that treatment with nitric acid gave mucic acid, while Bente (14) isolated laevulic acid on heating the sea-weed with mineral acid. Haedicke, Bauer and Tollens (15) isolated 2 grams of galactose from 500 grams of the sea-weed, and work by Müther and Tollens (16) indicated the presence of a small proportion of pentose or methylpentose. Lintner, Düll and Kiermayer (17) considered that the isolation of hydroxymethylfurfural-phenylhydrazone proved the presence of fructose, and Sebor (18) designated Carragheen as a complex carbohydrate containing galactose, glucose and fructose residues with a small quantity of pentose. In 1921 Haas and Hill (19) reported the presence of two polysaccharide ethereal sulphates in Irish moss, and Haas (20) later published/

published a method of separating the two, which depended on the fact that one fraction , the Cold Extract (C.E.), was readily soluble in cold water, while the other, the Hot Extract (H.E.), was only soluble in hot water. Both C.E. and H.E. had high ash contents, not reduced on prolonged dialysis, and H.E. was shown to be chiefly a calcium polysaccharide ethereal sulphate.

Russell-Wells (21) showed that an ethereal sulphate formula applied also to C.E., and she reported the presence of sodium, potassium and traces of iron and magnesium in the ash of both C.E. and H.E., with the H.E. always containing more calcium, and less sodium and potassium, than the C.E. A further publication by Haas and Russell-Wells (22) described attempts to remove the sulphate residues by treatment with dilute acid, without breaking down the carbohydrate complex. These were unsuccessful, due to the fact that the conditions necessary to effect the separation of sulphate from H.E. involved the complete breakdown of the rest of the molecule. These workers also confirmed the presence of fructose and glucose in H.E. Fructose was deemed to be present on the basis of a positive Seliwanoff test, while glucose was identified as glucosazone, after the ketose had been destroyed with hydrochloric acid, and as potassium hydrogen saccharate, after the mucic acid, formed from the galactose had been removed. Attempts to remove

the sulphate residues with alkali were also unsuccessful, only 20% removal being recorded in 16 hours with 3% sodium hydroxide at 110°.

M.R. Butler (23), working with Irish moss collected in Eastern Canada, obtained somewhat different results for the inorganic constituents from those of Haas and his school. Her "standard extract" contained a relatively high percentage of potassium with a low calcium content, while the 2:1 ratio for the sulphate after hydrolysis to the sulphate in the ash, demanded by the Haas ethereal sulphate formula, did not hold, the ratio being nearer 3:1. However, by dialysing against the appropriate salts, Butler prepared the pure potassium and calcium salts, for which the 2:1 ratio held. She explained the high ratio in the original extract by assuming that it was a mixture of the potassium, calcium and ammonium ethereal sulphates.

Dillon and O'Colla (24) have reported the acetolysis of carragheen extract with acetic anhydride and sulphuryl chloride. They obtained two sulphate-free acetates, composed exclusively of galactose units. It is evident, however, from the method of preparation and from the facts that the two acetates are sulphate-free and yield galactose exclusively on hydrolysis, that considerable degradation has taken place.

Buchanan, Percival and Percival (25) have subjected the/

the Chondrus polysaccharides to the methylation process, which leaves little doubt as to the structure and mode of linkage of the repeating anhydrogalactose sulphate unit. Analyses of the inorganic constituents were in good agreement with those reported by Haas. C.E. was shown to be chiefly the potassium-sodium ethereal sulphate, with a relatively low calcium content; $[\alpha]_D^{18^\circ}$ + 50°; ash (as sulphate), 22.4%; sulphate in the ash, 12.1%; total sulphate, 35.1%. H.E., on the other hand, was chiefly the calcium salt with small amounts of sodium and potassium; $[\alpha]_D^{18^\circ}$ + 63°; ash (as sulphate), 18.7%; sulphate in the ash, 12.5%; total sulphate, 23.8%. Hence, the 1:2 ratio of sulphate in the ash to sulphate after hydrolysis holds for H.E., but not for C.E. Percival claims that the physical differences between C.E. and H.E. can be attributed to the difference in calcium content. This view of the essential identity of C.E. and H.E., apart from the mineral constituents, was strengthened by the fact that treatment of a gel of H.E. with sodium oxalate gave a solution of low viscosity, while dialysis of C.E. against calcium chloride gave a calcium salt with properties similar to H.E. (26).

Hydrolysis of the extracts with acid confirmed galactose as the main constituent. This sugar, estimated as galactosemethylphenylhydrazone, was present to the extent of 34% in C.E. and 37% in H.E. Although the/

the galactose-free syrup from H.E. yielded both glucosazone and β-methylglucoside tetra-acetate on suitable treatment, the yields indicated that glucose can form only a small part of the galactose-free syrup. No evidence for the existence of glucose in the galactose-free syrup from C.E. was obtained, but colorimetric estimations indicated the presence of 20-22% of a ketose in this product.

Since neither extract could be acetylated by the usual methods, direct methylation with methyl sulphate and caustic potash, according to Bell (27), was resorted to. The process was tedious, due to the necessity for dialysis after each methylation, and in neither case did the methoxyl content ever reach that demanded by a dimethyl hexose ethereal sulphate. However, very little degradation was assumed to have taken place, as the methylated products still retained the ethereal sulphate grouping.

Methylation of C.E. proceeded to OMe 14.5%, and the hydrolysis mixture was shown to contain both 2:6-dimethyl and 2-monomethyl galactose. Methylated H.E. (OMe, 14.2%) contained the same mixture of sugars. Although no information was obtained by the methylation process regarding the non-galactosic portion of the molecule, the isolation of 2:6-dimethyl galactose gave considerable insight into the mode of linkage and the location/

location of the ethereal sulphate group in the repeating galactose units. The argument may be summarised as follows:-

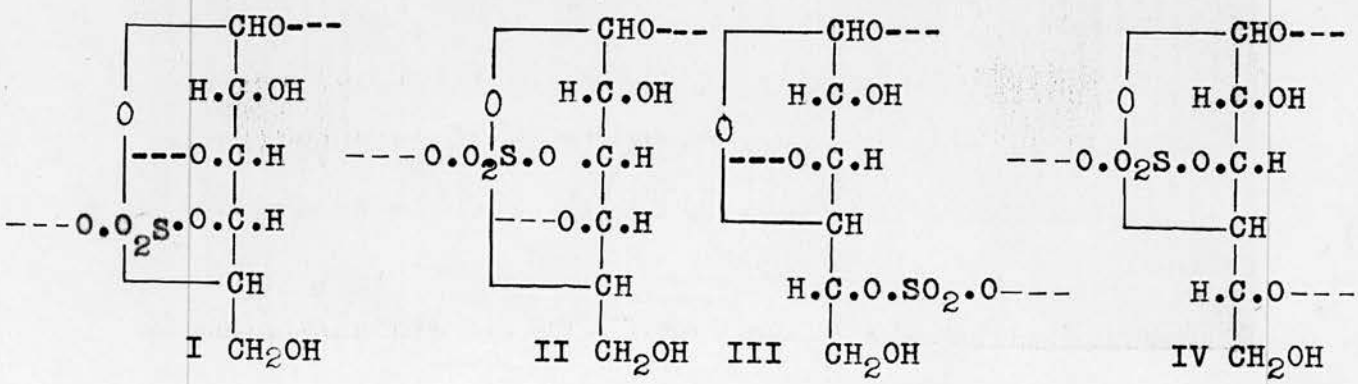
1. C_2 and C_6 are regarded as free in the polysaccharides.

2. The units are probably pyranose in character, since the rate of hydrolysis of C.E. and H.E. with acid was not in agreement with the presence of galactofuranose units.

3. The ethereal sulphate is probably located in C_4.

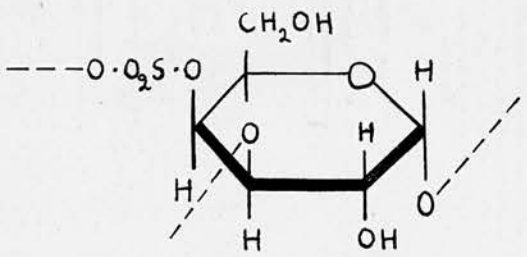
This assumption was based on the fact that the rate of removal of sulphate by caustic soda (4%) was extremely slow, taking 73 hours to remove 80%. This confirmed the earlier work of Haas and Russell-Wells (22).

It has been shown in a number of cases (28,29), that, where hydrolysis of hexose sulphates or toluenesulphonates is difficult, there is no possibility of interaction with another hydroxyl group to form an anhydro-ring. On the assumption that the hydroxyl groups on C_2 and C_6 are free in the repeating galactose sulphate residue, the possibilities are as follows:-



II and IV would hydrolyse with ease to give a 3:6-anhydrogalactose unit. III would hydrolyse to form an ethylene oxide ring, which, however, would be readily broken in the presence of aqueous alkali (30); furthermore, the presence of galactofuranose units is unlikely from the evidence obtained in 2. I, however, could only yield a 2:4- or 4:6-anhydrogalactose, products which have never been obtained from either sugar sulphates or toluenesulphonates.

On this evidence, the repeating anhydrogalactose sulphate unit in the carragheen polysaccharides has a 1:3-linkage, a linkage which is now common in galactans, with the ethereal sulphate on C_4:-



On account of the positive rotations of both C.E. and H.E., and their methylated derivatives, and the preponderance of d-galactose residues, the glycosidic linkage was assumed to be α.

Some progress into the constitution of the hitherto unidentified/

unidentified 'galactose-free syrup' has recently been reported by Young and Rice (31). They claim to have isolated 2-ketogluconic acid in considerable quantity. Their method was to hydrolyse the polysaccharide with oxalic acid and potassium oxalate in an atmosphere of nitrogen. The hydrolysis mixture was then taken to dryness and the residue extracted with alcohol, in which galactose is almost completely insoluble, and the alcohol-soluble material, on treatment with acetone and copper sulphate, gave a crystalline product, which was identified as diacetone-2-ketogluconic acid.

Further evidence for the presence of this acid was obtained by methylation. The methylated H.E. was obtained in crystalline form, m.p. 130-140°(decomp.); $[\alpha]_D^{18°}$ + 48.0°; OMe, 15.2%; ash, 18.2%. Hydrolysis of this product, followed by complete methylation and distillation, gave a fraction boiling at 140-165°/0.05mm. (OMe, 56.4%). This gave a crystalline amide with methanolic ammonia, which Young and Rice claimed to be 2:3:4:6-tetramethyl 2-ketogluconic acid amide. The presence of such an acid would certainly explain the claims, made repeatedly by earlier workers, for the existence of fructose in the carragheen polysaccharides.

Hassid (32) has obtained from the red alga, Irideae laminarioides, a polysaccharide ethereal sulphate, where the experimental difficulties appear to be less pronounced than in the other ethereal sulphates, and has/

has suggested a simple sodium galactan ethereal sulphate formula.

The plants, after being washed with water and alcohol, are extracted on the steam-bath, and the filtered extracts evaporated and precipitated in alcohol to yield a snow-white substance; $[\alpha]_D$ + 69.2°; ash, 25.4%; sulphate in the ash, 17.5%; total sulphate, 34.5-37.2%. The ash was shown to be chiefly sodium sulphate with small amounts of calcium and magnesium sulphates. The analysis shows that the 1:2-ratio of sulphate in ash to sulphate after hydrolysis holds for this polysaccharide, hence proving the existence of an ethereal sulphate group.

Hassid prepared the free galactan sulphuric acid by electrodialysis. The amorphous powder obtained, after concentration and precipitation in alcohol, had a pH of 2.86 for a 1% solution and an equivalent weight of 366, the theoretical equivalent of a pure galactan sulphuric acid, $C_6H_9O_4.O.SO_2.OH$, being 242. This high value was attributed to part of the sulphate being removed during dialysis. The original sodium salt gave a titration curve typical of a salt of a strong acid, indicating that the free galactan sulphuric acid was a strong acid.

Hydrolysis of the polysaccharide with acid showed galactose, identified as its phenylosazone, to be present/

present to the extent of 53.2%, calculated as anhydro-galactose. No other sugars could be isolated. Hassid reported that the sodium ethereal sulphate grouping could be removed, either with 0.5 N sulphuric acid or 5% baryta, to yield the pure galactan, without destroying the carbohydrate complex.

Acetylation with pyridine and acetic anhydride yielded a diacetate, which still retained the ethereal sulphate. Methylation of the sodium galactan sulphate by the method of Haworth and Learner (33), followed by two treatments with Purdie's reagents, yielded the dimethyl sodium galactan sulphate (OMe, 20.0%; $[\alpha]_D$ in $CHCl_3$, + 17.2°). Hydrolysis of this fully methylated compound gave a reducing, sulphate-free syrup, which did not crystallise. Glycoside formation yielded a non-reducing syrup, which distilled at 90°/0.1mm. to give a crystalline dimethyl methylgalactoside, the constitution of which, however, was not determined.

By methylating the sulphate-free galactan, prepared by acid hydrolysis of the original sulphuric ester, Hassid obtained a crystalline trimethyl galactan (OMe, 44.5%), hydrolysis of which yielded a syrup, which distilled at 94°/0.1mm. to give a non-crystalline trimethyl galactose. No osazone could be prepared from this sugar. Oxidation of this trimethyl galactose, first with bromine-water and then with nitric acid, followed/

followed by esterfication with 3% methanolic hydrogen chloride, gave a syrup, which Hassid described as a dimethyl arabodimethoxyglutarate. Hydrolysis of this ester yielded a dimethoxyhydroxyglutaric acid.

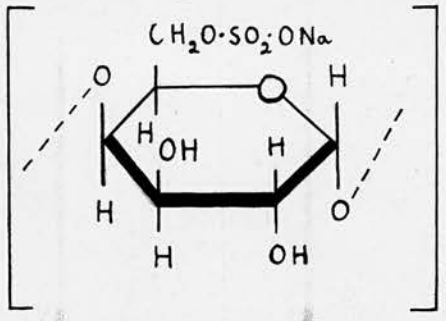
The fact that a dimethoxy compound was obtained proved, according to Hassid, that C_6 was occupied by a methoxyl group in the trimethyl galactan, for if C_6 were free, a trimethoxyglutaric acid should have been obtained, assuming a pyranose ring structure. From this evidence, Hassid rejected a 1:6-linkage between the galactose units.

The 1:2-linkage was excluded since no osazone could be prepared from the trimethyl galactose, formed by the hydrolysis of the fully methylated galactan, indicating that C_2 carried a methoxyl group.

Assuming the usual pyranose structure for galactose, Hassid rejected the 1:5-linkage, and also the 1:3-linkage, since such a linkage had not been discovered in a naturally ocurring substance at that time. On the other hand, the 1:4-linkage was common in many naturally occurring polysaccharides.

Finally, Hassid gave the probable position of the ethereal sulphate group as C_6, for steric reasons, and determined the molecular weight of the sodium galactan sulphate by the micro-chemical method of Rieche (34), thereby deducing that the chain length must be approximately/

approximately six sodium galactose sulphate units:-



n where n = 6 (approx.)

It is clear that this formula must be accepted with reserve for several reasons. Firstly, since 1935 the 1:3-linkage, and not the 1:4-linkage, has been shown to be common in polysaccharides containing galactose, e.g. agar (35), damson gum (36), gum arabic (37), and the carragheen polysaccharides (25). Secondly, the formation of osazones from hexoses containing a methoxyl group on C_2 has also been shown to be quite common, e.g. 4:6-dimethyl galactosazone from 2:4:6-trimethyl galactose (35), 6-monomethyl galactosazone from 2:6-dimethyl galactose (25,38), etc. The fact that an osazone cannot be prepared from a partially methylated sugar cannot be taken as evidence that C_2 is occupied by a methoxyl group. Thirdly, it seems remarkable that, although 5% baryta removes the ethereal sulphate group, methylation with methyl sulphate and caustic soda, in which process slight alkalinity is difficult to avoid, does not.

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PART I.

EXPERIMENTAL.

The sea-weed for this investigation was kindly supplied by Dr. A.P. Orr of the Marine Station, Millport.

Preparation of the Hot Extract (H.E.).

The air-dried material in 300-500g. lots was tied in muslin bags, and washed in running water for 10 days to remove salts and the Cold Extract as far as possible. The sea-weed was then repeatedly extracted with water on the steam-bath, until the extracts were no longer appreciably viscous. The combined extracts were filtered hot, concentrated at 50°/15mm. to small volume, and the brown viscous solution added drop by drop with mechanical stirring to alcohol to yield a white, fibrous solid. This was dehydrated with fresh alcohol, washed with ether, and dried in a vacuum desiccator.

Properties of H.E.

The H.E. was non-reducing to Fehling's solution and gave no acid reaction. $[\alpha]_D^{15^\circ} + 51^\circ$ (c,0.532 in water).

Inorganic Analysis Of H.E.

For all quantitative work the H.E. was purified by dialysing against running water for six days, filtering, and isolating as before as a white solid. It was dried over phosphorus pentoxide in a vacuum at 70-80° to constant weight.

Ash Determination.

H.E. (1.6408g) was incinerated in a platinum crucible, and the carbon removed by ignition in a full Bunsen flame to give an ash (0.2054g; 12.5%). Treatment with sulphuric acid to constant weight gave the ash as sulphate (0.2884g; 17.6%). In a second experiment H.E. (1.5186g) gave ash (0.2118g; 13.9%) and ash as sulphate (0.2632g; 17.3%).

Analysis of the Ash as Sulphate.

The ash as sulphate (0.25-0.30g) was dissolved completely in 1:1-concentrated hydrochloric acid: water (20ml.), and the solution made up to 100 ml. in a standard flask. Several of these standard ash solutions were prepared, and used for the following determinations:-

Sulphate was determined on 25ml. solution gravimetrically as barium sulphate (1). Sodium was determined on 20ml. as sodium zinc uranyl acetate (2). Potassium was determined on 25ml. as dipotassium monosodium cobaltinitrite (3). Calcium was determined on 50ml. as calcium oxalate by the single precipitation method (4). The calcium oxalate was then dissolved in dilute sulphuric acid, and the solution titrated with standard permanganate (5). After the removal of the calcium, magnesium was determined in the filtrate by precipitation with 8-hydroxyquinoline (6). The results/

results are tabulated below:-

	Ash.	H.E.(calc. from ash).
Calcium	20.7%	3.61%
Magnesium	5.6	0.99
Sodium	1.2	0.20
Potassium	0.6	0.10
Sulphate	72.7	12.70
	100.8	

The determinations of sulphate in the ash, obtained by direct ignition of H.E. in the absence of sulphuric acid, were always lower than the above, and varied considerably in different experiments. Two typical experiments yielded 9.11 and 8.34% sulphate in H.E., the sulphate being estimated after fusing the ash with a sodium peroxide-sodium carbonate mixture (7). An even lower result (7.72%) was obtained by dissolving the ash directly in hydrochloric acid, and hydrogen sulphide was evolved in appreciable amounts.

Total Sulphate.

H.E. (ca. 0.25g) was heated with 1:1-concentrated hydrochloric acid: water (20ml.) at the boiling-point for five hours, filtered, and the sulphate determined in the filtrate as barium sulphate (SO_4, 23.9%).

Hydrolysis of H.E. with 0.5 N Oxalic Acid in Air. Galactose Determination.

H.E. (4.983g) was hydrolysed at 100° with 0.5 N oxalic acid (210ml.) to constant rotation (26 hours) $[\alpha]_D^{15°}$ + 25° (c, 2.373 from the weight of H.E.). The solution was decolorised with charcoal, neutralised with calcium carbonate, filtered, and evaporated at 35°/15mm. to a syrup, admixed with calcium sulphate. This was extracted with small quantities of water, and the filtered extracts evaporated to a syrup (4.515g)

Galactose was determined quantitatively by dissolving the syrup in water (45ml.), adding ethanol (45ml.), glacial acetic acid (0.5ml.) and methylphenylhydrazine (3.75ml.), and leaving at 0° for 4 days. The crystalline galactose methylphenylhydrazone was filtered off, washed thoroughly with water, alcohol and ether, and dried in a vacuum over phosphorus pentoxide for 3 days (3.055g). The crystals, on recrystallisation from ethanol, gave m.p. 186-187°. Under the same conditions 3.219g. pure galactose ($C_6H_{12}O_6$) gave 4.978g. methylphenylhydrazone (m.p. 186°, without recrystallisation). Calculated amount of galactose ($C_6H_{12}O_6$) in H.E., 39.6%.

Preparation of the 'Galactose-Free Syrup'.

The filtrate and washings from the galactose methylphenylhydrazone precipitate were evaporated at 30°/15mm. to 20ml., and the remaining sugars recovered by/

by the method of Lüdtke (8). Ethanol (30ml.) and benzaldehyde (4ml.) were added, and the solution refluxed for 4 hours. The solution was kept at 0° overnight, the benzaldehyde methylphenylhydrazone filtered off, washed thoroughly with water, and the alcohol removed from the filtrate and washings by evaporation. The aqueous solution was washed twice with ether, decolorised with charcoal, and evaporated at 30°/15mm. to a glass (1.473g.), i.e. 29.6% of H.E.

In a second experiment H.E. (11.070g.) was hydrolysed with 0.5 N oxalic acid (400ml.) at 100° for 23 hours, neutralised with barium carbonate, and worked up as before to give a syrup (10.24g.), which gave galactose methylphenylhydrazone (6.829g), i.e. galactose in H.E., 39.9%. The filtrate yielded a 'galactose-free syrup' (3.07g), i.e. 27.7% of H.E. (This result is low through loss by accident).

Examination of the 'Galactose-Free Syrup'.

This was a hygroscopic glass, $[\alpha]_D^{15°} \pm 0.0°$ in water, giving the following reactions:-

1. It was reducing to Fehling's and Barfoed's reagents, and gave Schiff's test.

2. The Seliwanoff, Bredereck and selenium dioxide tests for a ketose were positive, and comparable with fructose.

3. There was no evidence of pentose or methylpentose by the ordinary colour reactions.

4. Uronic acid was shown to be absent by the naphtho-resorcin test.

5. The glass gave a copious precipitate of iodoform in the cold with alkaline hypoiodite.

6. The product, obtained by hydrolysis with oxalic acid and neutralisation with barium carbonate, was a barium salt.

Found: Ba, 20.8%; calc. equiv. of free acid, 262.

Attempted Preparation of the Free Acid.

The 'galactose-free syrup' (1.30g.) in water was treated with a slight deficiency of 0.1 N sulphuric acid, the barium sulphate filtered off, the filtrate decolorised with charcoal, and evaporated at 35°/15mm. to a syrupy glass. This was extracted with alcohol and the filtered extracts evaporated to a syrupy glass (0.63g.); $[\alpha]_D^{19^\circ}$ + 1.2°(c,2.1 in water.)

Found: equiv, 634; by titration with 0.025 N sodium hydroxide (phenolphthalein)..

Attempted Preparation of the Methyl Ester.

The 'galactose-free syrup' (1.30g.) was refluxed with 1% methanolic hydrogen chloride (50ml.) for 6 hours, barium chloride being precipitated. The solution was neutralised with barium carbonate, filtered and evaporated at 35°/15mm. to dryness. The residue was extracted with alcohol, and the filtered extracts evaporated to a syrup (0.90g.) which was non-reducing and/

and gave a negative iodoform test.

Found: OMe, 10.0%

The syrup (0.85g.) in methanol (5ml.) was methylated with Purdie's reagents to give a viscous syrup (0.22g.) which was not further investigated.

Hydrolysis of H.E. with 0.1 N Oxalic Acid in Nitrogen. (9).

H.E. (10.23g) was heated at 100° with a mixture of 0.1 N oxalic acid and 0.1 N potassium oxalate (400ml.) for 26 hours, in an atmosphere of nitrogen, to give $[\alpha]_D^{18°}$ + 24°(c, 2.558 from weight of H.E.). The solution was cooled, filtered, neutralised with potassium hydroxide (phenolphthalein) , and evaporated at 35°-40°/15mm. to dryness. The brown, syrupy residue was extracted with boiling 95% alcohol (2 litres), until the extracts were almost colourless, and the combined extracts evaporated to dryness. The residue was then extracted with absolute alcohol (1.5 litres), and the filtered extracts again evaporated. This extraction with absolute alcohol was twice repeated with 500ml. and 150ml. respectively to give a syrupy glass (2.08g), which was dried in vacuo over phosphorus pentoxide.

The glass was shaken with dry acetone (500ml.) and anhydrous copper sulphate (35g) for six days, filtered and the filtrate evaporated at 35°/15mm. to a syrup, containing a few crystals. This was treated with ether (150ml.) and 0.01 N sulphuric acid (10ml.), and the ether layer separated, dried over anhydrous

sodium sulphate, and evaporated to a syrup, which partially crystallised (fraction E; 0.89g.). The aqueous layer was then extracted with chloroform (300ml.), the chloroform extracts dried over anhydrous sodium sulphate, and evaporated to a mobile syrup, containing a few crystals (fraction C; 0.35g.).

Long needle-shaped crystals (6mg) were isolated from fraction E by dissolving the syrup in ether, decanting, and recrystallising the residue from ether. They had m.p. 119° and were neutral to litmus.

Fractions E and C, both of which were acid to litmus, were combined, but on attempting to distil darkening set in. The dark-brown syrup was extracted with ether, and the solvent removed to yield a syrup (0.78g), which solidified overnight. Methyl ester formation was carried out by dissolving in methanol (10ml.), and treating with an ethereal solution of diazomethane for 24 hours. A neutral, mobile syrup was obtained on removal of the solvent, and this was distilled in a high vacuum in the presence of barium carbonate:-

Fraction.	Bath temp.	$n_D^{16°}$	Yield.
1.	125-130°/0.10mm.	1.4802	0.213g.
2.	130-185°/0.10mm.	1.4992	0.098g
			0.311g.

Nothing further distilled on raising the temperature to/

to 220°, and the residue in the flask was a black, charred mass.

Examination of Fraction 1.

This was a yellow liquid, $[\alpha]_D^{17°} \pm 0.0°$ (c, 0.960 in chloroform), which darkened on exposure to air. It gave Schiff's test, a red colour with aniline acetate, and decolorised neutral permanganate.

Found: OMe, 25.8%.

A solution of fraction 1 (0.08g) in water (2ml.) was saturated with sodium acetate, and treated with semicarbazide hydrochloride (0.5g.), but no solid semicarbazone was formed.

Hydrolysis of Sulphate from H.E. with N Sodium Hydroxide.

H.E. (1.521g.) was heated with N sodium hydroxide (200ml.) at 100° in the presence of barium chloride (1.533g). At definite intervals, 25ml. samples were withdrawn, water (25ml.) and dilute acetic acid (10ml.) added, and the solution centrifuged. The residual sulphate in 50ml. of the solution was then determined by hydrolysing with hydrochloric acid, adding barium chloride, and filtering off the barium sulphate on an ashless filter-paper. The sulphate hydrolysed was then obtained by subtracting the residual sulphate from the total sulphate (23.9% of H.E., i.e. 0.0910g. $BaSO_4$ in 50ml. sample):-

Period of Hydrolysis. (hours).	Residual Sulphate. (in g. of $BaSO_4$).	Sulphate Hydrolysed. (in g. of $BaSO_4$).	% Hydrolysis.
4	0.0702	0.0208	22.9
10	0.0656	0.0254	27.9
24	0.0546	0.0364	40.0
32	0.0474	0.0436	47.9
48	0.0406	0.0504	55.4
56	0.0380	0.0530	58.3
72	0.0344	0.0566	62.2

Preparation of the Acid of H.E.

H.E. (0.75g.) in water (50ml.) was treated with hydrochloric acid to bring the normality up to 1, and dialysed against N hydrochloric acid until free from calcium (9 days). The solution was then dialysed against distilled water until free from chloride (5 days), and made up to 250ml. in a standard flask. Since charring set in when a small quantity of this solution was taken to dryness and dried in a vacuum at 80°, this acid was not isolated in the solid state. The concentration of the solution was determined as follows:-

25ml. solution required 3.72ml. 0.05035 N sodium hydroxide for neutralisation (phenolphthalein), i.e. solution is 7.492×10^{-3} N. The neutralised solution was/

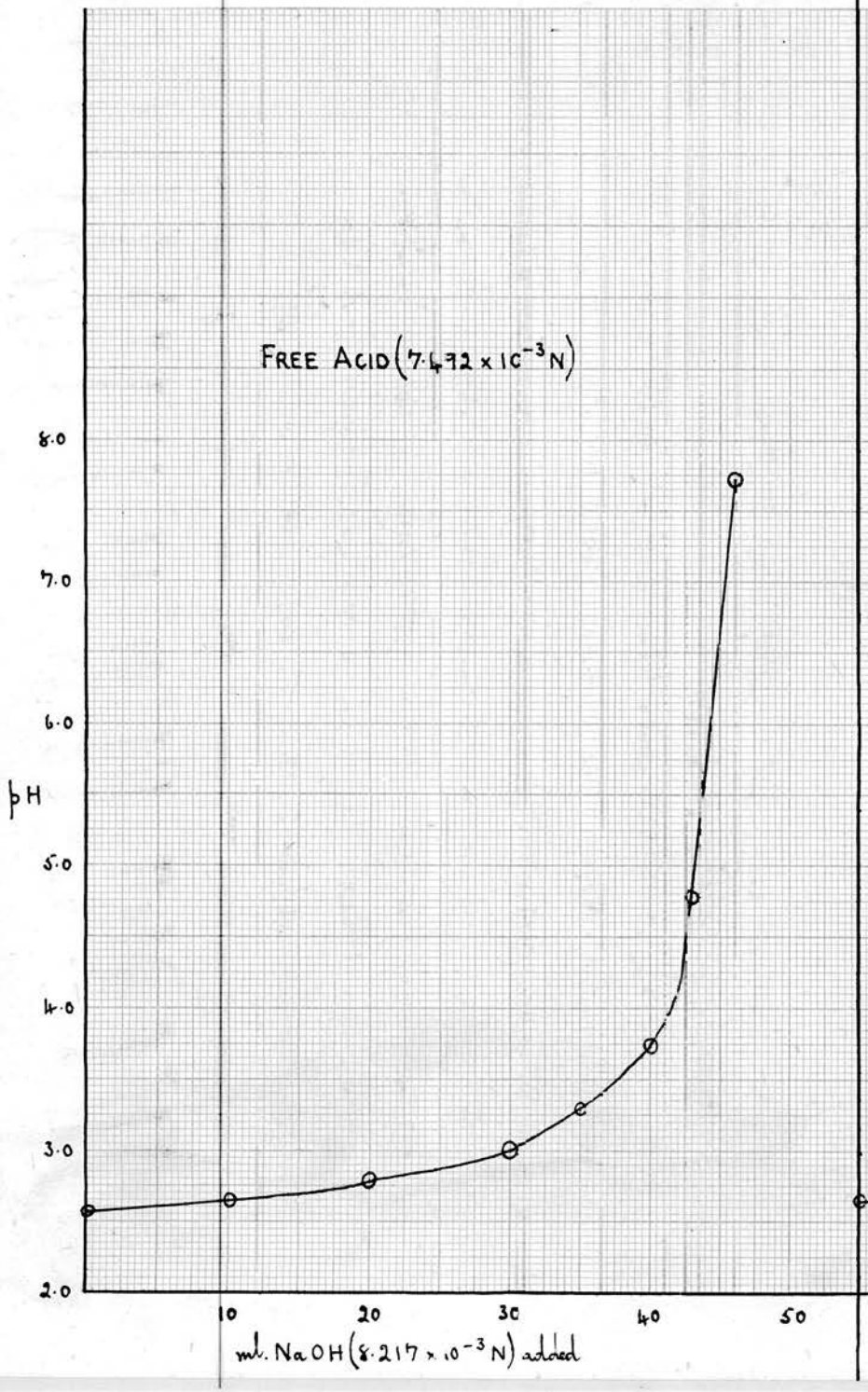
was then evaporated in vacuo to give the sodium salt (0.0736g), i.e. equiv. of sodium salt, 393; equiv. of free acid, 371. Hence the weight of acid in 250ml. solution, is 0.695g.

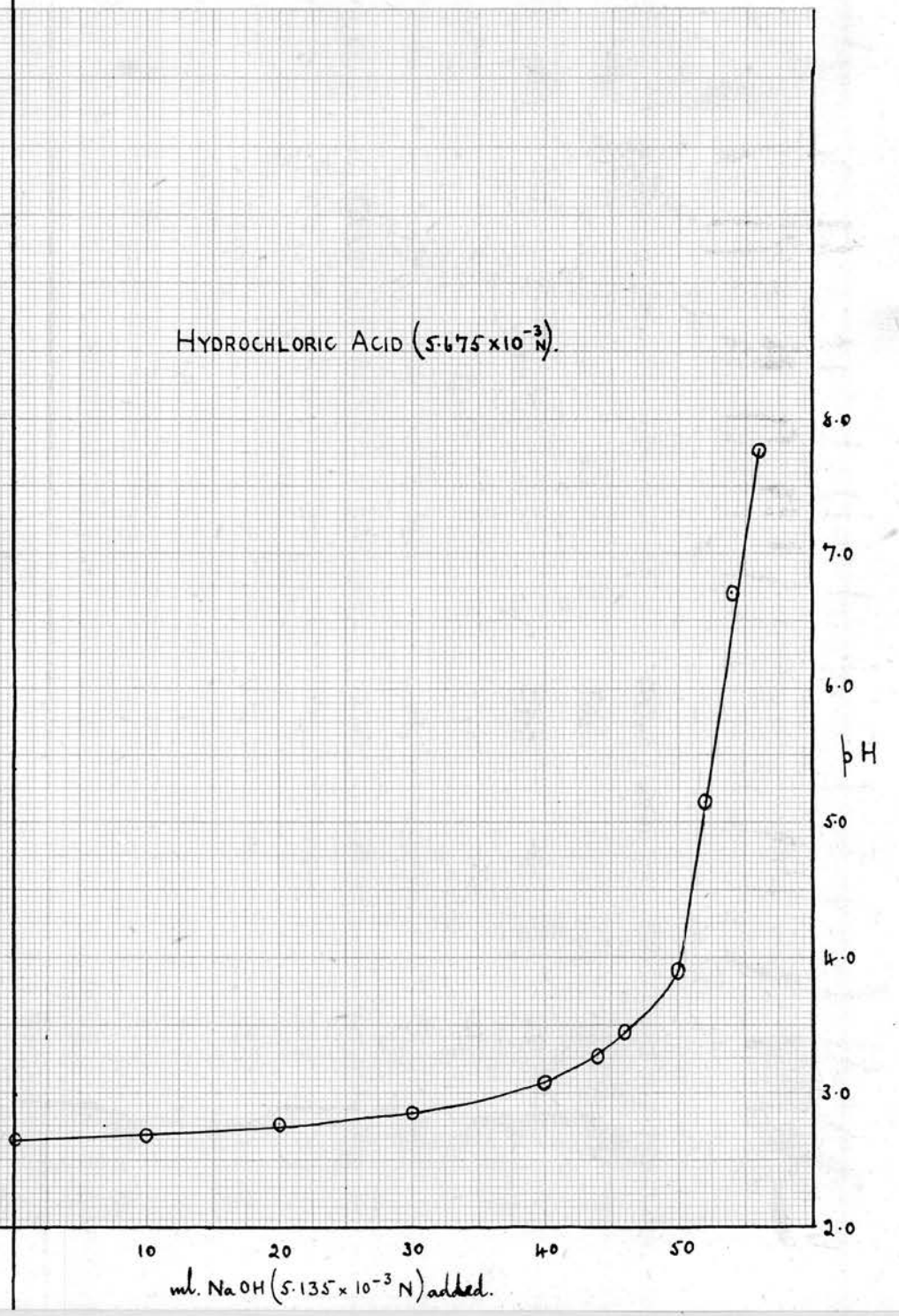
The acid was non-reducing, but became reducing on heating at 100° for 10 minutes.

$[\alpha]_D^{19°}$ + 43° (c, 0.139 in water); total sulphate, 25.9%.

Neutralisation Curve of the Free Acid.

Quinhydrone (0.5g.) was added to 50ml. acid solution in a beaker, and the solution mechanically stirred. A platinum electrode was immersed in the solution, and combined into a cell with the saturated calomel electrode, which formed the negative pole. The e.m.f. of the combination was determined directly by the Cambridge Portable Potentiometer, and the pH of the solution calculated (10). Sodium hydroxide (8.217×10^{-3} N) was then added from a burette, the pH calculated after each addition, and the neutralisation curve plotted. For comparison, the neutralisation curve of hydrochloric acid (5.675×10^{-3} N) against sodium hydroxide (5.135×10^{-3} N) was also plotted. The quinhydrone electrode is only applicable to solutions of pH below 8:-


FREE ACID (7.472×10^{-3} N)
pH
8·0
7·0
6·0
5·0
4·0
3·0
2·0
10
20
30
40
50
ml. NaOH (8.217×10^{-3} N) added


HYDROCHLORIC ACID (5.675×10^{-3} N).
pH
8·0
7·0
6·0
5·0
4·0
3·0
2·0
10
20
30
40
50
ml. NaOH (5.135×10^{-3} N) added.

Free Acid.			Hydrochloric Acid.		
NaOH added	e.m.f.	pH	NaOH added	e.m.f.	pH
-	308mv.	2.58	-	304mv.	2.65
10ml.	304	2.65	10ml.	302	2.69
20	296	2.79	20	298	2.76
30	284	3.00	30	292	2.86
35	270	3.24	40	280	3.07
40	242	3.73	44	268	3.28
43	182	4.77	46	258	3.45
46	12	7.71	50	232	3.90
			52	160	5.15
			54	70	6.71
			56	8	7.78

Oxidation of H.E. with Periodate.

Periodic Acid Uptake (11).

Finely powdered H.E. (0.6360g) in water (20ml.) was treated with 0.54M periodic acid (20ml.), and thoroughly mixed. 5ml. solution were then removed, diluted with water to 10ml, neutralised with sodium bicarbonate (1.5g.), 30ml. approximately 0.1 N sodium arsenite, containing 20g. sodium bicarbonate per litre, and 20% potassium iodide (1 ml.) added, and left at room temperature for 10-15 minutes. The excess arsenite was then titrated with 0.1014N iodine:-

Period of Oxidation.	Vol. 0.1014N iodine.	Difference.	HIO_4 uptake per 0.0795g H.E.
15 mins.	12.65ml.	-	-
7 hours	13.41	0.76ml.	0.00739g.
1 day	14.08	1.43	0.01391
3	14.93	2.28	0.02218
5	15.68	3.03	0.02947
7	16.32	3.67	0.03572
11	17.22	4.57	0.04446

The oxidation, still incomplete, had to be discontinued for lack of solution.

If the molecular weight of the repeating unit in H.E. be taken as 400 (equiv. of sodium salt, 393; page 35), the periodic acid uptake after 11 days is 1.17mols. per repeating unit.

Formic Acid formed with Sodium Metaperiodate (12).

Finely powdered H.E. (0.7032g) in water (20ml.) was treated with 0.54M periodic acid (20ml.) and a slight deficiency (20.50ml.) of approximately 0.5 N sodium hydroxide, so that the resulting solution was faintly acid to methyl red. 5 ml. solution were then withdrawn, diluted with water to 200ml, methyl red (2ml.) added, and titrated with 0.01009 N sodium hydroxide:-

Period of Oxidation.	Vol. 0.01009 N NaOH.	Difference.	H.COOH formed per 0.05812g H.E.
30mins.	2.6ml.		
8 hours	2.6		
1 day	2.6		
2	3.9	1.3ml.	0.000603g.
5	5.3	2.7	0.001253
8	5.9	3.3	0.001531
11	6.6	4.0	0.001857
15	7.0	4.4	0.002043
20	7.7	5.1	0.002367
33	9.4	6.8	0.003156

For a repeating unit of 400, the formic acid formed after 33 days corresponds to 0.47mol. per repeating unit.

DISCUSSION.

After a preliminary washing with cold water, Gigartina stellata yielded a non-reducing polysaccharide ethereal sulphate on extraction with hot water, $[\alpha]_D^{15^\circ}$ + 51° in water. As with the Chondrus crispus extracts, this has been termed the Hot Extract (H.E.). The Cold Extract was not investigated.

The H.E. gave all the characteristic reactions of an ethereal sulphate, outlined on pages 2-4:-

1. No precipitate of barium sulphate was obtained on adding barium chloride to an aqueous solution of the H.E. until after hydrolysis. This showed the sulphate group was not ionised.

2. An immediate precipitate of calcium oxalate was obtained on adding ammonium oxalate to an aqueous solution of the H.E., indicating that the calcium was ionised.

3. Determinations of the ash content, obtained by direct ignition of the H.E. in the absence of sulphuric acid, varied considerably, but the ash as sulphate was a constant (17.5%), not reduced by prolonged dialysis. Analysis of the ash as sulphate showed it to consist of calcium (20.7%), magnesium (5.6%), sodium (1.2%), potassium (0.6%), and sulphate (72.7%). As in the case of Chondrus crispus, the H.E. was chiefly the calcium salt.

4. The total sulphate content of the H.E. (23.9%) was approximately/

approximately double the sulphate content of the ash as sulphate (12.7%). As indicated on page 4, concordant results for sulphate in the ash could only be obtained by determining the ash as sulphate. The ash, obtained by direct ignition of the H.E. in the absence of sulphuric acid, gave hydrogen sulphide on treatment with acid, indicating that reduction to sulphide had taken place.

The identification of the sugars comprising the carbohydrate portion of the molecule was greatly hindered by the fact that one constituent at least was extremely unstable to acid reagents. Hydrolysis in aqueous solution invariably led to considerable decomposition, even with very dilute acids. On complete hydrolysis with 0.5 N oxalic acid (page 29) and neutralisation with barium carbonate, a syrup was obtained, which was found to contain d-galactose to the extent of 39.8% of H.E. After the removal of the galactose as methylphenylhydrazone the 'galactose free syrup' was recovered by the method of Lüdtke (8), the yield of which was about 30% of H.E.

This 'galactose-free syrup' was optically inactive, and contained barium (20.8%). It was therefore a salt. It gave the Seliwanoff, Bredereck and selenium dioxide tests for a ketose, and Fehling's, Barfoed's and Schiff's tests were all positive. Pentose, methylpentose and uronic acid, however, appeared to be absent on the evidence of the usual colour reactions. On treatment

with/

with alkaline hypoiodite in the cold, a precipitate of iodoform was obtained.

These reactions of the 'galactose-free syrup' were not inconsistent with a barium salt of a ketohexonic acid, and this view became more feasible when Young and Rice (13) reported the isolation of 2-ketogluconic acid from Chondrus crispus. Complete failure, however, to identify this 'ketohexonic acid', or even to secure any additional evidence for the presence of such an acid, must be admitted, and all the succeeding work on this portion of the molecule was chiefly negative in character. An attempt to prepare the free acid (page 30) from the barium salt by treatment with sulphuric acid gave a product, which appeared to be an acid but had a much higher equivalent than that demanded by the barium content of the salt, while treatment with methanolic hydrogen chloride gave a non-reducing syrup, the methoxyl content (10.0%) of which was lower than that required by one, far less two, methoxyl groups in the molecule. Moreover, this syrup was almost completely destroyed on methylation with Purdie's reagents.

The possibility did exist that the 0.5 N oxalic acid used to ensure the complete hydrolysis of H.E. had decomposed, to a greater or less extent, the 'galactose-free syrup', so that some of the above reactions/

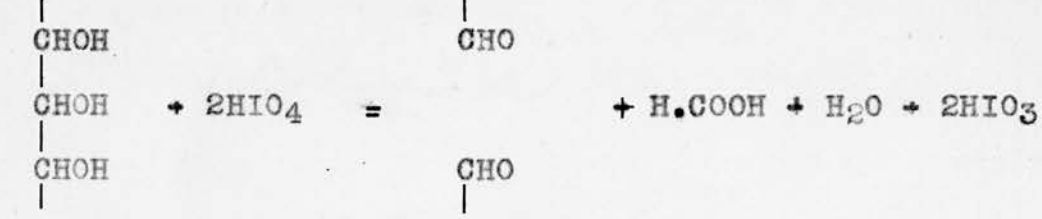
reactions might be due to decomposition products. For example, laevulic acid, which has been identified in the hydrolysis products of methylated H.E., would account for the iodoform reaction, while furfural, or some of its derivatives, would give Schiff's test. In the isolation of 2-ketogluconic acid from Chondrus crispus, Young and Rice used 0.1 N oxalic acid in nitrogen. Hence the hydrolysis of H.E. was repeated using the exact conditions of Young and Rice (9), (page 31). An alcohol-soluble residue was obtained in 20% yield, and this with acetone gave a mobile acid, which darkened on attempting to distil in a high vacuum. After esterification with diazomethane, only a small quantity (3% of H.E.) distilled, while the boiling-points and refractive indices indicated that even this was a decomposition product. Its properties suggested it was a derivative of furfural, such as ω -methoxy-5-methyl-furfural, although this particular compound readily forms a crystalline semicarbazone.

As in the case of Chondrus crispus, the rate of removal of sulphate from H.E. by aqueous sodium hydroxide (4%) was slow, only 62% being removed in 72 hours, thus indicating a 'straight' hydrolysis, without anhydro-ring formation. The full significance of this will be discussed in Part 2.

The free acid of H.E. could be prepared by prolonged dialysis/

dialysis against hydrochloric acid. It had an equivalent of 371, which is in fairly good agreement with the value of 351, calculated from the analysis of the metals. The neutralisation curve was similar to that of hydrochloric acid, indicating that the free acid was a typical strong acid. The acid was unstable, and decomposed on heating the aqueous solution at 100°.

H.E. was shown to be attacked by periodic acid, thus indicating the presence of α-glycol linkages in the polysaccharide, but the reaction was so slow that it is difficult to give any quantitative significance to the results. However, if the molecular weight of the repeating unit in H.E. be taken as 400, from a consideration of the equivalent of H.E., then one repeating unit has taken up 1.17 mol. periodic acid in 11 days and liberated 0.47 mol. formic acid in 33 days. Since both the oxidations were incomplete, these correspond approximately to two molecules periodic acid and one molecule formic acid, which would suggest that each repeating unit contained three consecutive hydroxyl groups:-



But in view of the slowness of the reaction, this argument must be accepted with reserve. The statement that H.E. did not react with periodic acid (14) was based/

based on a reaction time of 2 days, which would normally be sufficient for the oxidation of a cis-glycol group, starch (15) and alginic acid (16) being completely oxidised in 24 hours.

SUMMARY.

1. The Hot Extract (H.E.) from *Gigartina stellata* has been shown to be chiefly a calcium-magnesium salt of a polysaccharide ethereal sulphate.
2. The sulphate group strongly resisted hydrolysis by aqueous alkali.
3. The free acid, prepared by dialysis, had an equivalent of 371, and was a typical strong acid.
4. H.E. was attacked slowly by periodic acid, both periodate being taken up and formic acid being liberated.
5. Complete hydrolysis of H.E. and neutralisation gave d-galactose (40%) and a salt of a carbohydrate acid (30%), which was not an uronic acid but gave the reactions of a ketose.

Attempts to identify this fraction were unsuccessful.

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PART 2.

EXPERIMENTAL.

Acetylation of H.E.

The H.E. was given a preliminary treatment with pyridine by the method employed by Pacsu and Mullen (1) for starch.

H.E. (15.88g.) was dissolved in water (200ml.), pyridine (700ml.) added, and the pyridine-water azeotrope distilled off at 50°/15mm. To ensure the complete elimination of water, the distillation was continued until the volume of the distillate was about 250ml. The H.E. was obtained in the form of a brown jelly in pyridine. Pyridine was added to bring the volume to 300ml., followed by acetic anhydride (200ml.) slowly with shaking, the flask being cooled in water, when the H.E. was precipitated as a fine, white solid. After 12 days at room temperature the acetate was centrifuged down, washed thoroughly with pyridine, alcohol and ether, and dried in a vacuum desiccator to yield a white powder, which gave $[\alpha]_D^{18°}$ + 46° (c, 0.820 in water).

Acetyl Determination.

A small quantity of the acetate was purified by dialysis against distilled water (4 days). To about 0.1g. of purified acetate, 25ml. of 0.1 N sodium hydroxide were added, and allowed to stand overnight. The excess alkali was titrated with 0.1 N sulphuric acid/

acid, using phenolphthalein (CH_3CO, 19.1%).

Preparation of Methylated H.E.

To the solution of the acetate in water (300ml.) methyl sulphate (120ml.) and 30% caustic soda (300ml.) were added in one twelfth portions every half hour with mechanical stirring, the temperature being kept below 20°C. The solution was then heated to 80° for half an hour, cooled, neutralised with glacial acetic acid, and dialysed against running water until free from sulphate (10 days). The dialysed solution was evaporated at 45°/15mm. to a brown glass (OMe, 16.2%). This glass was taken up in water (100ml.), worked up with pyridine as described above, and reacetylated to give CH_3CO, 5.9%. Simultaneous deacetylation and methylation as before gave OMe, 17.2%. This was followed by a third acetylation (CH_3CO, 4.4%) and methylation (OMe, 17.6%), and a fourth acetylation (CH_3CO, 3.5%) and methylation (OMe, 18.2%).

Finally the dialysed solution was concentrated to about 200ml., and added drop by drop to alcohol to give methylated H.E. as a light-brown, fibrous solid (13.97g), $[\alpha]_D^{15°}$ + 43° (c,1.570 in water).

Attempts to Increase the Methoxyl Content.

1. By Direct Methylation- Methylated H.E. (13.97g; OMe, 18.2%) was dissolved in water (150ml.), and methyl sulphate (100ml.) and 30% sodium hydroxide (250ml.) added/

added in one tenth portions every 10 minutes at 45°. This was followed by heating to 80°, neutralisation and dialysis as above. The concentrated solution was given two further methylations under the same conditions to yield a methylated H.E. of lower methoxyl content (13.07g; OMe, 16.5%). Acetylation of this product gave CH_3CO, 6.2% and OMe, 16.9%.

2. By the Thallium Method (2). Methylated H.E. (0.50g; OMe, 16.5%) was dissolved in water (10ml.), N thallous hydroxide (2.5ml.) added, and the solution evaporated at 40°/15mm. to dryness. The cream-coloured glass was ground to a powder, and boiled with methyl iodide (45ml.) until it no longer gave an alkaline reaction (15 days). The methyl iodide was distilled off, the solid suspended in water, centrifuged, and the solution evaporated to a glass, which still contained thallous iodide. This was extracted with small quantities of cold water, centrifuged, and evaporated to a glass (0.31g; OMe, 17.4%).

Inorganic Analysis of Methylated H.E.

Methylated H.E. (1.0850g; OMe 18.6%) gave ash as sulphate (0.1978g; 18.2%). Analysis of the ash as sulphate gave the following:-

	Ash	Methylated H.E.(calc. from ash)
Calcium	20.6%	3.75%
Magnesium	5.2	0.94
Sodium	1.7	0.31
Sulphate	70.4	12.80
	97.9.	

The total sulphate was 24.7%.

Hydrolysis of Methylated H.E. with 0.5 N Oxalic Acid.

Methylated H.E. (5.133g; OMe 18.6%-) was hydrolysed at 100° with 0.5N oxalic acid (200ml.) to constant rotation (26 hours); $[\alpha]_D^{15^\circ}$ + 37° (c, 2.567 from the weight of methylated H.E.). The solution was neutralised with calcium carbonate, and worked up to give a syrup admixed with calcium sulphate. This was extracted with alcohol, and the filtered extracts evaporated at 35°/15mm. to a brown syrup (4.002g; OMe, 26.3%).

Glycopyranoside Formation.

The syrup was refluxed with 6% methanolic hydrogen chloride (160ml.) until non-reducing (8 hours), neutralised with silver carbonate, filtered and evaporated at 40°/15mm. to dryness. The residue was extracted with ether, and the extracts evaporated to a syrup (2.336g OMe, 36.0%; $n_D^{11^\circ}$ 1.4665), which was distilled in a high vacuum:-

Fraction/

Fraction	Bath temp.	$n_D^{10°}$	Yield.
1.	100°/0.10mm.	1.4295.	0.089g.
2.	145-155°/0.05-0.10mm.	1.4737.	1.893
			1.982.

Identification of Fraction 1 as Methyl Laevulate.

This was a colourless, mobile liquid, $n_D^{10°}$ 1.4295.

Preparation of the 2:4-Dinitrophenylhydrazone.

To the liquid (0.080g) in ethanol (2ml.) a well shaken suspension of 2:4-dinitrophenylhydrazine in ethanol (5ml.) was added. The mixture was heated, a few drops conc. hydrochloric acid added, and boiled for 30 seconds. On cooling an orange-red solid (0.027g) separated, which gave m.p. 135-136° after two recrystallisations from a mixture of chloroform and petrol-ether (b.p. 40-60°); mixed m.p. with authentic 2:4-dinitrophenylhydrazone (m.p. 137°) from methyl laevulate, 134-135°.

Identification of Fraction 2 as 2:6-Dimethyl Methylgalactopyranoside.

Fraction 2 was a pale yellow syrup; $n_D^{10°}$ 1.4737; $[\alpha]_D^{12°}$ + 69.8° (c, 1.430 in water).

Found: OMe, 40.4.

Calc. for $C_9H_{18}O_6$; OMe, 41.9%.

Complete Methylation and Isolation of Tetramethyl d-Galactopyranose Anilide.

Fraction 2 (0.567g) was dissolved in a mixture of acetone (10ml.) and water (10ml.), and methylated with methyl sulphate (15ml.) and 30% sodium hydroxide (35ml.), added in one tenth portions every 10 minutes at 56°. The solution was then heated at 75° for half an hour, cooled, and extracted with chloroform (500ml.). The extracts were washed once with water (100ml.), dried over anhydrous sodium sulphate, and evaporated at 40°/15mm. to a syrup, which was remethylated under the same conditions.

The resulting brown syrup was then methylated with Purdie's reagents. The syrup was dissolved in methyl iodide (25ml.), and silver oxide (10g) added in one fifth portions every hour, the flask being kept at 47° with occasional shaking for 7 hours. The solution was filtered, the residues exhaustively extracted with chloroform, and the filtrate and extracts evaporated to a syrup, which was remethylated.

The brown, mobile syrup (0.469g) distilled at 90-110°/0.02mm., in the presence of barium carbonate, to yield a colourless liquid (0.396g; $n_D^{11°}$ 1.4495), which was hydrolysed with N sulphuric acid (15ml.) at 100° for 6 hours. The acid was neutralised with barium carbonate, the contents heated at 100° for a further hour, filtered, and the filtrate evaporated at 35°/15mm./

35°/15mm. to a yellow, viscous syrup (0.353g).

This was dissolved in ethanol (7.5ml.), freshly distilled aniline (0.2ml.) added, and refluxed at 85-90° for 2 hours. On standing overnight, a white solid (0.233g) was obtained, which on recrystallisation from ethanol gave m.p. 193-194°, mixed m.p. with authentic tetramethyl d-galactopyranose anilide (m.p. 191°) 190°, and $[\alpha]_D^{15°}$ -80.0° (c, 0.600 in acetone).

Hydrolysis and Isolation of a Crystalline Dimethyl Galactose.

Fraction 2 (0.814g) was hydrolysed with N sulphuric acid (30ml.) to constant rotation (5 hours); $[\alpha]_D^{14°}$ + 82.4° (c, 0.716).

The solution was neutralised with barium carbonate, heated at 100° for 1 hour, filtered, and evaporated at 35°/15mm. to give a crystalline product. The highest m.p. obtained for this sugar was 119-120°, after three recrystallisations from dry ethyl acetate. It crystallised in large needles, showing $[\alpha]_D^{15°}$ + 48.0° (10mins.) ⟶ + 87.1° (240 mins; constant; c, 0.666 in water).

Found; C,46.5; H,7.5; OMe, 29.1.

Calc. for $C_8H_{16}O_6$; C,46.2; H,7.7; OMe, 29.8%

This sugar was also obtained in the form of large plates by recrystallisation from a mixture of ethyl acetate and petrol-ether (b.p. 40-60°).

Isolation of 6-Methyl Galactosazone from the Dimethyl Galactose.

The free sugar (0.336g) in water (5ml.) was treated with a filtered solution of phenylhydrazine hydrochloride (0.4g), crystalline sodium acetate (2.0g) and sodium bisulphite (0.2g) in water, and heated at 100° for 3 hours to give an impure osazone. Further yields were obtained on heating the filtrate (total yield, 0.253g). The product, after 3 recrystallisations from ethanol, gave yellow needles, m.p. 201-203°, and mixed m.p. with authentic 6-methyl galactosazone (m.p. 201-203°), 198-200°.

Found: OMe, 7.1

Calc. for $C_{19}H_{24}O_4N_4$: OMe, 8.3%

Glycoside Formation on the Dimethyl Galactose.

The sugar in 1% methanolic hydrogen chloride (c, 1.675) showed the following changes in $[\alpha]_D^{13°}$:- + 41.8° (45 mins.), + 23.2° (18hrs.), + 12.5° (25hrs), -9.5° (42hrs.), -15.5° (48hrs.), -28.7° (66hrs.), -38.8° (92hrs.) -43.0° (114hrs; constant).

Evidence for the Presence of a Methoxyl Group on C_6.

1. Method of Oldham and Rutherford (3)-Fraction 2 (0.281g) in pyridine (2ml.) was treated with p-toluene sulphonyl chloride (0.85g). After 5 days at room temperature the dark-brown solution was poured into a mixture of ice and water. The solution was thoroughly extracted/

extracted with benzene (200ml.), and the benzene extracts washed with dilute hydrochloric acid, aqueous sodium bicarbonate and water, dried overnight over anhydrous sodium sulphate, and evaporated at 40°/15mm. to a yellow syrup (0.754g). $[\alpha]_D^{15°}$ + 32°(c, 2.346 in chloroform).

Found: OMe, 15.5.

Calc. for $C_{23}H_{30}O_{10}S_2$: OMe, 17.6%

The syrup (0.748g), in dry acetone (15ml.), was heated in a sealed tube with anhydrous sodium iodide (0.75g) at 100° for 2 hours. The contents of the tube were filtered, and the precipitated sodium p-toluene sulphonate washed with acetone to give 0.0164g., indicating that 6.0% of fraction 2 had an unsubstituted C_6. (Calc. yield from 0.748g. dimethyl methylgalactoside ditoluene-p-sulphonate, with a tosyl group on C_6, is 0.274g. sodium p-toluene sulphonate). The acetone solution was taken to dryness under reduced pressure, the residue taken up in a mixture of chloroform (50ml.). and water (50ml.), and excess iodine removed with sodium thiosulphate. The chloroform layer was separated, the aqueous layer thoroughly extracted with chloroform, and the chloroform solution dried over anhydrous sodium sulphate. Removal of the solvent yielded a light brown syrup.

The syrup was treated with silver nitrate (0.75g) and acetonitrile (10ml.), and the solution refluxed for

2/

2 hours. The solvent was completely removed at 100°/15mm., and the residue repeatedly extracted with boiling benzene, which was decanted off and evaporated at 85°/15mm. to a brown syrup (0.722g.). The silver salts were dried at 100°, treated with a boiling mixture of 1:1- fuming nitric acid: water (50ml.), diluted to 300ml., and boiled for half an hour to give only a negligible quantity of silver iodide.

2. Periodic acid method (4)- The sugar (23.4mg) in water (2ml.) was treated with N sodium bicarbonate (2ml.) and 0.3 M periodic acid (2ml.). The solution was mixed and allowed to stand at room temperature for 1 hour. Then were added in turn with thorough mixing N hydrochloric acid (3ml.) and 1.2 M sodium arsenite (2ml.). When the precipitate and yellow colour had completely disappeared, M sodium acetate (2ml.) and dimedon reagent (1ml.) were added, and allowed to stand at room temperature for 3 days. The crystalline precipitate was filtered off, washed thoroughly with water, and dried in a current of air at 85°-95° for 20 minutes:-

Yield, 39.8mg., i.e. 1.21 mol. (calc. as CH_2O) per mol. of $C_8H_{16}O_6$.

M.p. 105-106°.

Under the same conditions, galactose (26.2mg) gave a white precipitate (17.2mg.), m.p. 183-185°, i.e. 0.40 mol. CH_2O per mol. of $C_6H_{12}O_6$. Reeves quotes m.p. 189-190° for the formaldimedon complex.

Derivatives of 2:6-Dimethyl Galactose.

2:6-Dimethyl Galactonic Acid.

The dimethyl galactose (1.586g) in water (25ml.) was treated with bromine (1.75ml.) at room temperature until non-reducing (6 days). Excess bromine was removed by aeration, the solution neutralised with silver carbonate, filtered, and the silver precipitated by passing hydrogen sulphide. The solution was filtered and evaporated at 40°/15mm. to a colourless viscous syrup (1.66g), which crystallised slowly on standing. After 2 months the crystalline mass was extracted with cold acetone to remove the syrup, and the crystals isolated as long needles, m.p. 139-140°, $[\alpha]_D^{17°}$ + 26.2° (c, 1.565 in water).

Found: C, 42.5; H, 7.1; OMe, 24.8; equiv., 241, by titration with 0.01 N NaOH (phenolphthalein).

Calc. for $C_8H_{16}O_7$: C, 42.9; H, 7.2; OMe, 27.7; equiv., 224.

Calc. for $C_8H_{16}O_7.H_2O$: C, 39.7; H, 6.7; OMe, 25.6; equiv., 242.

2:6-Dimethyl γ-Galactonolactone.

The crystalline acid (0.963g) distilled at 180°/0.02mm. to yield a light brown, viscous syrup (0.896g), which gave $n_D^{18°}$ 1.4760 and $[\alpha]_D^{17°}$ - 48.8° (initial) ⟶ - 23.9° (28 days, still incomplete; c, 1.086 in water).

Found: OMe, 28.6; equiv., 205, by titration with 0.025 N NaOH (phenolphthalein).

Calc. for $C_8H_{14}O_6$: OMe, 30.1%; equiv., 206.

The titration was typical of a γ-lactone, the indicator colour appearing almost immediately on adding the sodium hydroxide, and then fading slowly. The end-point was marked by a permanent colour on heating to 50°.

2:6-Dimethyl Galactonamide.

The syrupy lactone (0.338g) was treated with methanolic ammonia (5ml.) at 0° for 2 days, and the solvent removed in a vacuum desiccator to give a crystalline amide (0.365g). After 3 crystallisations from ethanol, it gave small needles, m.p. 154-155°, $[\alpha]_D^{16°}$ + 46.1° (c, 0.846 in water).

Found: C, 43.5; H, 7.3; N, 6.0; OMe, 26.7.

Calc. for $C_8H_{17}O_6N$: C, 43.1; H, 7.7; N, 6.3; OMe, 27.8%.

The amide gave a negative Weerman reaction. To the amide (0.101g) in water (2ml.) was added a solution (2ml.) of 6% sodium hypochlorite, and kept at 0° for 3 hours. Excess hypochlorite was destroyed with sodium thiosulphate solution, semicarbazide hydrochloride (0.2g. in 2ml. water) added, and the solution saturated with sodium acetate. No appreciable quantity of precipitate was formed after 20 hours. In a parallel experiment with gluconamide (0.102g), a copious white precipitate of hydrazodicarbonamide was formed within 5 minutes.

Phenylhydrazide of 2:6-Dimethyl Galactonic Acid.

The lactone (0.204g) was allowed to react with phenylhydrazine (1 mol.) in boiling ether for 15 minutes. On removing the solvent and heating at 85-90° for 2 hours, crystals were obtained (0.250g), which were thoroughly washed with ether. Recrystallisation from ethanol-ether gave white needles, m.p. 140° alone and in admixture with authentic 2:6-dimethyl galactonic acid phenylhydrazide (m.p. 139-140°), supplied by Dr. Bell.

Found: C, 53.1; H, 7.2; N, 8.9; OMe, 19.0.

Calc. for $C_{14}H_{22}O_6N_2$: C, 53.5; H, 7.1; N, 8.9; OMe, 19.8%.

2:6-Dimethyl β-Methylgalactoside.

The dimethyl galactose (0.505g) in pyridine (10ml.) was treated with acetic anhydride (5ml.) at room temperature for 3 days. The brown solution was poured into water (150ml.), extracted with chloroform (200ml.), the chloroform extracts washed with dilute sulphuric acid, aqueous sodium bicarbonate and water, and dried over anhydrous sodium sulphate. Removal of the solvent yielded a crystalline acetate (0.80g).

This product in glacial acetic (2ml.) was treated with glacial acetic acid saturated with hydrogen bromide (3ml.) at 0°. After 3 hours chloroform (20ml.) was added, the solution poured on to ice, more chloroform (30ml.) added, and the chloroform layer separated. The chloroform/

chloroform solution was then washed thoroughly with water, aqueous sodium bicarbonate and water, and dried over anhydrous sodium sulphate, all reagents being cooled in ice. After removal of the chloroform, the acetobromo-compound was obtained as a yellow syrup.

The syrup in dry methanol (40ml.) was shaken with silver carbonate (3g) for 24 hours, filtered, and evaporated at 35°/15mm. to a non-reducing syrup (0.66g), $[\alpha]_D^{18°}$ + 18.9° (c, 6.6 in chloroform).

The syrup was deacetylated by dissolving in acetone (25ml.) and treated with excess 0.1 N sodium hydroxide (60ml.) at room temperature for 3 hours. The solution was neutralised with acetic acid, and evaporated at 40°/15mm. to dryness. The residue was extracted with chloroform (100ml.), the extracts dried over anhydrous sodium sulphate, the chloroform removed under diminished pressure, and the syrup distilled at 145-150°/0.05-0.07mm. to yield a yellow distillate (0.376g), $n_D^{19°}$ 1.4763, which crystallised on standing. After 2 recrystallisations from a mixture of ether and petrol-ether (b.p. 40-60°), hygroscopic needles were obtained, m.p. 71-72°, $[\alpha]_D^{19°}$ -21.6° (c, 0.924 in chloroform).

Found: OMe, 41.0%.

Calc. for $C_9H_{18}O_6$: OMe, 41.9%.

2:6-Dimethyl 3:4-Monoacetone β-Methylgalactoside.

The ether-petrol-ether mother liquors from the above/

above crystallisations were evaporated to a syrup (0.231g), which crystallised slowly. This was dissolved in dry acetone (50ml.) and shaken with anhydrous copper sulphate (2g) for 4 days. The solution was filtered, and evaporated to a yellow syrup, which crystallised after a few minutes, and gave the iodoform reaction after treatment with acid. This product was extracted with petrol-ether (b.p. 40-60°), and the extracts evaporated to give small needles, m.p. 52-55°. Recrystallisation was impossible, due to the small quantity of material available and its high solubility in the common organic solvents. An attempt was made to purify the product by distillation, the fraction boiling at 100-110°/0.10mm. being collected (0.101g) and crystallising immediately. $[\alpha]_D^{19°}$ + 4.0° (c, 1.01 in chloroform).

Found: OMe, 34.5.

Calc. for $C_{12}H_{22}O_6$: OMe, 35.5%.

2:6-Dimethyl Galactose Anilide.

The dimethyl galactose (0.313g) in ethanol (7ml.) was allowed to react with aniline (1 mol.) at 80° for 3½ hours to give a crystalline anilide in quantitative yield. After 3 recrystallisations from ethanol, white needles were obtained, m.p. 121-122°, $[\alpha]_D^{17°}$ + 15.1° (c, 0.664 in ethanol).

Found: C, 56.7; H, 7.8; N, 5.2; OMe, 22.2.

Calc. for $C_{14}H_{21}O_5N$: C, 59.4; H, 7.5; N, 4.9; OMe, 21.9%.

The preparation of the anilide was repeated, but the elementary analysis could not be improved.

Hydrolysis of Methylated H.E. with 0.5N Oxalic Acid, followed by Furanoside Formation.

Methylated H.E. (5.231g; OMe, 18.6%) was hydrolysed with 0.5 N oxalic acid as before to give a syrup (3.537g), which was treated with 2% methanolic hydrogen chloride (100ml.) at room temperature. After 8 days the solution was non-reducing, and had $[\alpha]_D^{17^\circ}$ --30° (c, 0.707). The solution was neutralised with silver carbonate, filtered, and evaporated at 40°/15mm., in the presence of barium carbonate, to dryness. The residue was extracted with ether, and the ether removed to give a syrup (2.753g), which was distilled in the presence of barium carbonate:-

Fraction	Bath temp.	$n_D^{16^\circ}$	Yield.
1	100°/15mm.	1.4302	0.049g.
2	120-145°/0.04-0.07mm.	1.4672	1.977g.
3	145-165°/0.02-0.03mm.	1.4710	0.298g.
4	165-205°/ "	1.4813	0.126g.
			2.450g.

Examination of Fraction 2.

This was a pale yellow syrup, $n_D^{16^\circ}$ 1.4672.

Found: OMe, 37.7;

Calc. for $C_9H_{18}O_6$: OMe, 41.9%.

Fraction 2 was redistilled on account of this low methoxyl content.

Fraction	Bath temp.	$n_D^{17°}$	Yield.	%OMe	$[\alpha]_D^{16°}$
2a	130-135°/0.03-0.05mm.	1.4673	1.318g.	39.0	-35.1
2b	135-145°/ "	1.4685	0.367g.		(c, 3.850 in water).
			1.685g.		

Isolation of 2:6-Dimethyl β-d-Galactose.

Fractions 2a and 2b were combined (1.665g), and heated with 0.1 N sulphuric acid (60ml.) at 100° to constant rotation (7½ hours); $[\alpha]_D^{17°}$ + 75.0° (c, 2.774). The solution was neutralised with barium carbonate in the usual way to give a syrup (1.658g), which crystallised on standing. The product was extracted with boiling ethyl acetate (150ml.), the extracts filtered to remove an insoluble residue, and the solvent removed in a vacuum desiccator to give the crystalline sugar (1.187g), m.p. 105-110° without recrystallisation.

Examination of Fraction 4.

This was a light brown syrup $n_D^{16°}$ 1.4813, $[\alpha]_D^{17°}$ -8.3° (c, 1.08 in water).

Found: OMe, 33.9%.

Attempted Osazone Formation.

Fraction 4 (0.108g) was heated with 0.1 N H_2SO_4 (16ml.) at 100° to constant rotation (6 hours); $[\alpha]_D^{17°}$ + 68.2° (c, 0.675). The solution was treated with a filtered solution of phenylhydrazine hydrochloride (0.4g), crystalline sodium acetate (2.3g) and sodium bisulphite (0.2g) in water, at 100° for 3½ hours to give a dark brown osazone. Attempts to purify this osazone failed.

Hydrolysis of Methylated H.E. with Methanolic Hydrogen Chloride.

Methylated H.E. (4.655g; OMe, 20.2%-) was refluxed with 1.3% methanolic hydrogen chloride (200ml.) for 24 hours, in the presence of anhydrous barium chloride (5.1g). $[\alpha]_D^{17^\circ}$ + 26° (c, 2.328 from the weight of methylated H.E.), after 2½ hours, did not change appreciably during the hydrolysis, and the solution darkened only slightly. The acid was neutralised with barium carbonate, the solution filtered and evaporated at 40°/15mm. to dryness. The residue was extracted with alcohol, and the filtered extracts evaporated to a hard hygroscopic, non-reducing syrup (4.098g), which still contained sulphur.
Found: OMe, 33.5%.

The above syrup (4.08g) was again refluxed with 2% methanolic hydrogen chloride (100ml.) for 16 hours, in the presence of barium chloride; $[\alpha]_D^{16^\circ}$ + 27° (initial) ⟶ + 23° (15½ hours; c, 4.08). The solution was neutralised with barium carbonate as above to give a syrup, which was extracted with boiling ether (300ml.), and the solvent removed to yield a light brown syrup (1.15g; fraction 1). The residue was extracted with alcohol, and the solvent removed to yield a syrupy glass (2.58g; fraction 2.).

Fraction 2 was further treated with 2.7% methanolic hydrogen chloride (100ml.) as above for 24 hours;

$[\alpha]_D^{15^\circ}$ + 28° (initial) ⟶ + 21° (22½ hours; c, 2.58). Neutralisation with barium carbonate gave a syrup, which gave an ether-soluble fraction 3 (0.23g) and an alcohol-soluble fraction 4 (2.13g).

Examination of the Ether-soluble Fractions 1 and 3.

Fractions 1 and 3 were combined (1.27g), and distilled in the presence of barium carbonate:-

Fraction	Bath temp.	$n_D^{13^\circ}$	Yield.	%OMe.
A	100°/15mm.	1.4330	0.006g,	
B	135-140°/0.03-0.05mm.	1.4740	0.708g.	38.0.
C	140-155°/0.04-0.07mm.	1.4757	0.401g.	38.2.
			1.115g.	

Hydrolysis of Fractions B and C and Isolation of 2:6-Dimethyl β-d-Galactose.

Fractions B and C were combined (1.04g), and hydrolysed with N sulphuric acid, considerable decomposition taking place. A syrup was obtained (0.85g) on neutralisation and evaporation, which crystallised on standing. Extraction with boiling ethyl acetate, followed by filtration and removal of the solvent in a vacuum desiccator, gave the crystalline sugar as before.

Examination of the Ether-insoluble Fraction 4.

This was a hygroscopic, non-reducing, syrupy glass. Found: OMe, 32.0%.

Fraction 4 in 0.5 N sulphuric acid (c, 0.668) gave $[\alpha]_D^{17^\circ}$ + 32.9°, constant at room temperature. At 100°, $[\alpha]_D^{18^\circ}$ showed the following changes:- + 47.9° (30 mins.), + 56.9° (1¾ hours), + 59.9° (5¼ hours).

Complete Methylation and Distillation.

Fraction 4 (1.75g) in methanol (5ml.) was methylated with Purdie's reagents. After 5 methylations a mobile syrup was obtained, which was distilled:-

Fraction	Bath temp.	$n_D^{17^\circ}$	Yield.	%OMe.
D	90°-95°/0.02-0.03mm.	1.4481	0.794g	55.6
E	95°-100°/ "	1.4478	0.544g	54.9
F	100-125°/ "	1.4483	0.117g	53.3
Residue.			0.136	
			1.591	

Attempted Removal of the Galactose Portion by Anilide Formation. (5).

Fraction D (0.427g) in N sulphuric acid (15ml) showed an increase in rotation at room temperature; $[\alpha]_D^{19^\circ}$ + 29.9° (15 mins.) ⟶ + 37.6° (48 hours; constant; c, 2.846). The solution, which was now reducing, was heated at 100°:-$[\alpha]_D^{19^\circ}$ + 40.8° (45 mins.) ⟶ + 27.0° (6 hours). The solution was decolorised with charcoal and neutralised with barium carbonate to give a yellow, viscous syrup. (0.29g).

The syrup in ethanol (7ml.) was treated with aniline (0.12g) at 80° for 3 hours to give an anilide (0.140g), which on recrystallisation from ethanol gave white needles/

needles, m.p. 194-195°, $[\alpha]_D^{16°}$ -77.6° (c, 0.696 in acetone).

After the removal of the crystalline tetramethyl galactose anilide, the syrupy residual anilide was decomposed with 0.2 N sulphuric acid (10ml.) at 100° for 3 hours. The solution was extracted with chloroform (20ml.), neutralised with barium carbonate, filtered, and evaporated at 35°/15mm. to 15ml. This solution was again extracted with chloroform (10ml.) to remove aniline and evaporated to a brown syrup, which partially crystallised (0.06g). This was found to be a barium salt, $[\alpha]_D^{16°}$ + 18.3° (c, 0.6 in water).

Isolation of a Further Quantity of this Barium Salt.

Fractions D, E and F were combined (0.90g) and treated with N sulphuric acid (15ml.) at room temperature; $[\alpha]_D^{16°}$ + 38.4° (30mins.) ⟶ + 42.5° (48 hours; constant; c, 6.020). The reducing solution was neutralised with barium carbonate, to give a syrup, admixed with a glass (0.77g). This was dissolved in water (20ml.), extracted with chloroform to remove tetramethyl methylgalactoside, and evaporated to a glass (0.15g).

This glass was reducing and gave a positive iodoform reaction in the cold. $[\alpha]_D^{16°}$ + 21.1° (c, 1.42 in water). Found: OMe, 19.8; Ba, 18.2%.

The chloroform extracts, containing the tetramethyl methylgalactoside, gave a colourless liquid (0.63g) on removal of the solvent. The glycosidic methoxyl was removed/

removed with N sulphuric acid at 100° in the usual way to give a syrup (0.51g), which distilled at 120-130°/0.02mm. to give a pale yellow distillate (0.40g). Treatment with aniline gave an anilide (0.225g), which on recrystallisation from ethanol gave white needles, m.p. 196-197°, $[\alpha]_D^{18°}$ -81.0°, (c, 0.654 in acetone).

Hydrolysis of Methylated H.E. with 4% Methanolic Hydrogen Chloride in Nitrogen.

Methylated H.E. (5.40g; OMe, 20.2%) was hydrolysed with 4% methanolic hydrogen chloride in the presence of anhydrous barium chloride as before, in an atmosphere of nitrogen, to yield a viscous syrup (4.77g). An attempt to purify this syrup (0.95g) by distillation gave a low yield:-

Fraction	Bath temp.	$n_D^{12°}$	Yield.
1	145-155°/0.06-0.07mm.	1.4750	0.293g.
2	155-195°/ " "	1.4738	0.093g.
			0.386g.

The yield, b.p. and refractive index suggest that only the dimethyl methylgalactoside in the syrup has distilled. The residue was a charred, dry mass.

The remainder of the syrup was purified by dissolving in water (40ml.), and treating with charcoal at room temperature. After 3 days the solution was filtered and evaporated at 30°/15mm. to a pale yellow syrup (3.58g).

Evidence for the Presence of an Ester Group in the Syrup.

The apparatus consisted of two test-tubes (8" x 1"), connected by glass tubing passing through rubber stoppers, with the inlet tubes dipping into the foot of each. The first test-tube contained exactly 10ml. 0.1 N sodium hydroxide, while the second contained about 10ml. water and acted as a trap. A tube from the latter led into the Zeisel apparatus.

The syrup (0.216g) was weighed out and placed in the first test tube. Nitrogen was bubbled through the apparatus, and the two test-tubes were maintained at 90°-95° until no further precipitate was formed in the alcoholic silver nitrate flasks (4 hours). The excess sodium hydroxide in the first test-tube was then titrated with standard sulphuric acid, and the precipitate of silver iodide weighed.

Found: COOMe, 8.4 (by weighing the silver iodide).

COOMe, 4.9% (by titration).

Hydrolysis of the Syrup with 0.17 N Barium Hydroxide in Nitrogen.

The syrup (3.36g) was treated with 0.17 N barium hydroxide (30ml.) at 40-45° for 6 hours in an atmosphere of nitrogen. The solution was neutralised with carbon dioxide, filtered, and evaporated at 30-35°/15mm. to a viscous syrup (3.32g). This syrup was dissolved in alcohol (20ml.), and added slowly to ether (200ml.), when/

when a white solid was precipitated and centrifuged down (0.35g; fraction A). The ether solution, on removal of the solvent, gave a yellow syrup (2.88g), which still contained sulphate (SO_4, 3.7%) but no barium.

This syrup was again treated with 0.17 N barium hydroxide at 50° for 8 hours as before to yield a syrup (2.94g), which on dissolving in alcohol (2ml.) and adding to benzene (100ml.) gave a precipitate (0.40g; fraction B). The benzene solution on evaporation gave a syrup (2.50g; fraction C).

Examination of Fraction A.

This was a cream coloured, hygroscopic, non-reducing powder, which became strongly reducing after heating with hydrochloric acid at 100° for 10 minutes. It gave a negative iodoform reaction. $[\alpha]_D^{16°}$ + 15.8° (c, 1.11 in water).

Found: OMe, 12.1; Ba, 29.8; SO_4, 23.0%.

Hydrolysis with Hydrochloric Acid.

Fraction A (0.11g) was heated at 100° with 0.3 N hydrochloric acid (12ml.) for 10½ hours in nitrogen; $[\alpha]_D^{16°}$ + 17.0° (30 mins) ⟶ + 14.2° (7½ hours, constant). The barium sulphate formed during the hydrolysis was filtered off and weighed (39.4mg, i.e. 14.6% SO_4 in fraction A). The yellow, reducing, solution was neutralised/

neutralised with silver carbonate, filtered, and hydrogen sulphide passed through the solution. The silver sulphide was filtered off, and the filtrate evaporated at 35°/15mm. to a white glass (0.037g). This glass, which was not completely soluble in water, was neutral to litmus and still contained barium and sulphate. It was strongly reducing, and gave a positive iodoform test, and the Seliwanoff, Bredereck and selenium dioxide tests for a ketose.

Found: OMe, 10.6%.

Examination of Fraction B.

This was a hygroscopic, non-reducing glass. It gave a negative iodoform reaction. $[\alpha]_D^{15°}$ + 17.2° (c, 4.07 in water).

Found: OMe, 19.5; Ba, 14.5; SO_4, 21.1%.

Examination of Fraction C.

Fraction C (2.293g) was distilled in a high vacuum:-

Fraction	Bath temp.	$n_D^{13°}$	Yield	%OMe.
D	140-145°/0.04-0.05mm.	1.4747	1.662g.	37.4.
E	residue.	1.4820	0.600g.	34.0.
			2.262	

At 145° the residue darkened and the pressure increased, so the distillation was stopped.

Chromatographic Adsorption of Fraction D (6).

Fraction D (1.60g) was dissolved in 1:1-chloroform-petrol-ether (b.p. 40-60°) and introduced into a column of alumina (35cm x 1cm) (Savory and Moore Ltd, London; standardised according to Brockmann), suspended in the same solvent. The filtrate was collected at the rate of one drop per second under gentle suction, and the fraction isolated by removal of the solvent under diminished pressure. The column was washed first with the same solvent, then pure chloroform and finally alcohol:-

Fraction	Volume of filtrate	Yield.	$n_D^{16°}$
1	25ml.	0.019g	
2	10	0.475	1.4731
3	10	0.300	1.4730
4	10	0.099	1.4730
5	10	0.146	1.4731
6	60	0.215	1.4732
7	60	0.267	1.4728
8	40	0.234	1.4732
		1.755	

The 8 fractions were combined, and redistilled at 140-145°/0.05mm., the distillate being collected in 2 portions:-

Fraction	$n_D^{18°}$	Yield.	%OMe.
D_1	1.4725	0.738g.	39.3.
D_2	1.4733	0.644	
		1.382.	

Fractions D_1 and D_2 were combined, and hydrolysed with N sulphuric acid as before to give crystalline 2:6-dimethyl β-d-galactose (0.92g), m.p. 109-112° on crystallisation from ethyl acetate.

Chromatographic Adsorption of Fraction E.

Fraction E (0.60g) was dissolved in 1:1-chloroform-petrol-ether (b.p. 40-60°), and chromatographed as described above. The following separation was effected:-

Fraction	Volume of filtrate	Yield	$n_D^{13°}$	%OMe	$[\alpha]_D^{17°}$
1	50ml.	0.189g.	1.4778		
2	30	0.172	1.4738	40.0	
3	65	0.225	1.4830	33.7	+ 39.0° (c, 2.0 in chloroform).
		0.586			

Complete Methylation of Fraction 3.

Fraction 3 (0.20g) was methylated 7 times with Purdie's reagents and the resulting syrup distilled:-

Fraction	Bath temp.	$n_D^{14°}$	Yield	%OMe.
3a	95-115°/0.03-0.04mm.	1.4500	0.094g	52.5
3b	115-160°/ " "	1.4497	0.027	
			0.121	

Fractions 3a and 3b were combined (0.11g) and heated with 0.1 N sulphuric acid (10ml.) at 100° for 8 hours, $[\alpha]_D^{18°}$ + 55.5° (initial) remaining almost constant during the hydrolysis. The reducing solution was neutralised with barium carbonate, filtered, and evaporated at 35°/15mm. to a glass (0.09g), which contained barium:-

Found: OMe, 31.2%

Attempted Recovery of the Methyl Ester.

The above glass (80mg) was refluxed with 2% methanolic hydrogen chloride (10ml.) for 8 hours, barium chloride being precipitated. The solution was neutralised with silver carbonate, filtered, and evaporated to a syrup (60mg). This was methylated 3 times with Purdie's reagents, and the product distilled at 0.05mm. to yield a colourless syrup (29mg).

Attempted Preparation of the Amide.

The above ester was treated with methanolic ammonia (1ml.) at 0° for 44 hours, and the solvent removed in a vacuum desiccator to give a syrup, which did not crystallise alone or on nucleation with tetramethyl fructofururonamide.

DISCUSSION.

Direct methylation of H.E. proved to be slow (OMe, 14.6% after 4 methylations), but the H.E. could be readily acetylated in the cold after a preliminary treatment with pyridine. This method was introduced by Pacsu and Mullen (1) for preparing esters and ethers of starch. Simultaneous deacetylation and methylation of this acetate (CH_3CO; 19.1%) at room temperature yielded a partially methylated H.E., the methoxyl content of which was raised to 18-20% by three similar acetylation, deacetylation and methylation treatments. In different preparations intermediate products were obtained with slightly different acetyl and methoxyl values from those recorded on page 49, but the final methoxyl by this method always lay between 18 and 20%. Attempts to increase this methoxyl content by direct methylation with methyl sulphate and caustic soda at 45°, and by the thallium method, were unsuccessful, the former method actually giving a product of lower methoxyl value (16.5%).

The methylated H.E. was shown to have similar properties to the H.E. It gave $[\alpha]_D^{15^\circ}$ + 43° in water. The ash as sulphate was 18.2%, while analysis of the ash as sulphate gave calcium (20.6%), magnesium (5.2%), sodium (1.7%) and sulphate (70.4%). Again, the total sulphate content (24.7%) was approximately double the sulphate /

sulphate in the ash (12.8%), indicating that the ethereal sulphate formula still applies to the methylated H.E. It is significant that no calcium nor magnesium has been replaced during the methylation process, while the high yield of methylated H.E. and the analysis figures suggest that little or no degradation has taken place.

Complete hydrolysis of the methylated H.E. with 0.5 N oxalic acid in air, followed by glycopyranoside formation and distillation in a high vacuum, gave two fractions (page 51):-

Fraction 1 (1.7%); n_D^{100} 1.4295;

Fraction 2 (37.0%); n_D^{100} 1.4737; OMe, 40.4%

Fraction 1 was identified as methyl laevulate by the preparation of its crystalline 2:4-dinitrophenylhydrazone. Laevulic acid is a common decomposition product of carbohydrates, particularly hexoses, on treatment with acids.

Fraction 2 was identified as 2:6-dimethyl methylgalactopyranoside (α-β-mixture) on the following evidence:-

1. Complete methylation, followed by hydrolysis and treatment with aniline, gave tetramethyl d-galactopyranose anilide in good yield, indicating that C_5 did not carry a methoxyl group.

2. Hydrolysis of fraction 2 gave a crystalline dimethyl galactose/

galactose, m.p. 119-120°, $[\alpha]_D^{15°}$ + 48.0° (10 mins.)⟶ + 87.1° in water (240 mins. constant). The upward trend of the rotation proved that the sugar had the β-configuration.

3. This sugar, on osazone formation, gave a monomethyl osazone, showing that one of the methoxyl groups occupied C_2. On recrystallisation, this product gave pure 6-methyl galactosazone, proving that the sugar was 2:6-dimethyl-β-d-galactose.

This sugar was first synthesised by Oldham and Bell, (7), who gave m.p. 128-130° and $[\alpha]_D$ + 46.8°⟶ + 87.5° in water, but Bell (8), in his recent studies on 2:6-dimethyl galactose, has repeated this work, and now quotes m.p. 106-108°, $[\alpha]_D^{21°}$ + 48.2°⟶ + 88.0°, for the pure β-sugar. He attributes a high m.p. to the presence of a small amount of the α-form.

4. Further confirmation of the structure of this sugar came from the fact that, on glycoside formation at room temperature with 1% methanolic hydrogen chloride, a mixture of dimethyl methylgalactofuranosides was obtained, as shown by the final negative rotation, $[\alpha]_D^{13°}$ + 41.8°⟶ -43.0° (constant). This proved C_4 to be unsubstituted.

5. That C_6 carried a methoxyl group was shown in two ways. Firstly, fraction 2, on treatment with p-toluene sulphonyl chloride, formed a ditosyl derivative, which on/

on treatment with sodium iodide in acetone gave a yield of sodium p-toluene sulphonate indicating that 6.0% only of fraction 2 had an unsubstituted C_6. Suitable treatment of the syrup obtained from the acetone solution showed that a negligible quantity of iodine had entered the molecule. Secondly, the free sugar, on oxidation with periodic acid in the presence of sodium bicarbonate, gave no test for formaldehyde with dimedon. Reeves (4) has shown that hexopyranoses, in which the primary alcoholic group is free, yield formaldehyde in almost quantitative yield. A crystalline precipitate was actually obtained in this experiment, but neither the yield nor the melting point were in agreement with the formaldimedon complex.

Since Oldham and Bell (7) had only prepared a few derivatives, it seemed advisable that the characterisation of 2:6-dimethyl galactose should be carried a stage further. Some of this work has since been confirmed by Bell (8). On oxidation with bromine the dimethyl galactose gave a syrup, which crystallised slowly on standing. The crystalline acid gave m.p. 139-140°, $[\alpha]_D^{17^\circ}$ + 26.2° in water. The methoxyl and equivalent agreed with the monohydrate, while the carbon and hydrogen analysis indicated an anhydrous formula. This may be attributed tc the fact that the analysis for carbon and hydrogen was carried out after drying the crystals/

crystals for several weeks over phosphorus pentoxide in a vacuum desiccator. Distillation of this acid yielded a syrupy lactone, $[\alpha]_D^{17^\circ}$ -48.8° ⟶ -23.9° in water (28 days, still incomplete), the negative rotation and slow hydrolysis proving it to be a γ-lactone. This was further evidence that C_4 was unsubstituted. Treatment with methanolic ammonia yielded an amide, m.p. 154-155°, $[\alpha]_D^{16^\circ}$ + 46.1° in water, which gave a negative Weerman reaction, thus confirming the evidence from the osazone that C_2 carried a methoxyl group. The phenylhydrazide was also prepared, m.p. 140°, not depressed on admixture with a synthetic specimen supplied by Dr. Bell. The dimethyl galactose was converted into the β-methylgalactoside, m.p. 71-72°, $[\alpha]_D^{19^\circ}$ -21.6° in chloroform, and its 3:4-monoacetone derivative, m.p. 52-55°, $[\alpha]_D^{19^\circ}$ + 4.0° in chloroform. Bell (8) quotes m.p. 73-75°, $[\alpha]_D^{21^\circ}$ -24.0° for authentic 2:6-dimethyl β-methylgalactoside, and m.p. 55°, $[\alpha]_D^{19^\circ}$ -4.5° for the acetone derivative. The small quantity of material available made the purification of the latter compound difficult, with the result that the properties vary slightly from those recorded by Bell. A crystalline anilide was also readily formed, m.p. 121-122°, $[\alpha]_D^{17^\circ}$ + 15.1° in ethanol, but was found to be unstable. This has been confirmed by Bell.

Since the yield (37.0%) of dimethyl methylgalactoside from/

from methylated H.E. was in good agreement with the yield (39.8%) of galactose from H.E., 2:6-dimethyl galactose must be assumed to be the chief building unit in the molecule of methylated H.E. It must be admitted, however, that no evidence of the constitution of the non-galactosic residue was obtained from this experiment. From a consideration of the yields obtained at each stage, the somewhat drastic treatment with 6% methanolic hydrogen chloride during glycoside formation seems to have almost completely destroyed this portion of the molecule, for the yield dropped from 78% after hydrolysis to 46% after glycoside formation. In an attempt to improve this yield hydrolysis with 0.5 N oxalic acid was carried out as before, followed by glycoside formation with 2% methanolic hydrogen chloride at room temperature, to give an ether-soluble syrup in 53% yield (page 63). Distillation gave the following:-

Fraction 1 (0.9%); $n_D^{16^\circ}$ 1.4302.

Fraction 2 (37.8%); $n_D^{16^\circ}$ 1.4672; OMe, 37.7%.

Fraction 3 (5.7%); $n_D^{16^\circ}$ 1.4710.

Fraction 4 (2.4%); $n_D^{16^\circ}$ 1.4813; OMe, 33.9%.

Fraction 1 was assumed to be methyl laevulate. On account of the low methoxyl value fraction 2 was redistilled in an attempt to effect a separation. The distillate was collected in two fractions, but the boiling-points and refractive indices indicated that

the/

the two were almost identical. Removal of the glycosidic methoxyl gave crystalline 2:6-dimethyl β-d-galactose in good yield, proving that fraction 2 was essentially a mixture of dimethyl α-and β-methylgalactofuranosides.

Fraction 3 was not examined, but was considered to be chiefly dimethyl methylgalactoside, the slightly higher boiling-point and refractive index suggesting the presence of a certain amount of the pyranoside.

The refractive index and methoxyl value indicated that fraction 4 was different from fractions 2 and 3, but the probability that it was monomethyl methylgalactoside, containing some dimethyl methylgalactoside, seemed reasonable, since the specific rotation of -8.3° changed to $+ 68.2^{\circ}$ on hydrolysis. An attempt to prepare an osazone, however, failed. Monomethyl galactose has been isolated in considerable quantity from both the methylated H.F. and C.E. of Chondrus crispus (9), where methylation was difficult and the methoxyl content never rose above 15%. It would be expected, therefore, that the proportion of monomethyl galactose in the present case would be very much less, on account of the higher methoxyl content of the methylated polysaccharide employed. No evidence as to the nature of the non-galactosic residue was obtained from this experiment, the combined yields (45.9%), as methylglycosides, of fractions 2, 3 and 4 being but slightly/

slightly higher than would be expected on the basis of the previously estimated galactose content (39.8%) of the H.E.

Methanolic hydrogen chloride (1.3%) was next tried as an hydrolytic agent, barium chloride being present to prevent the formation of methyl sulphate during hydrolysis (page 65). Although this reagent caused but little decomposition, it did not remove the sulphate very rapidly. However, after three treatments, the hydrolysis mixture was separated into an ether-soluble portion (30%) and an ether-insoluble portion (46%). The former was distilled, and the distillate shown to be chiefly dimethyl methylgalactoside. The latter, which presumably contained the non-galactosic portion together with dimethyl methylgalactoside was fully methylated and distilled, but no separation could be effected. Two methods were used in an attempt to isolate this non-galactosic residue, both of which gave very poor yields:-

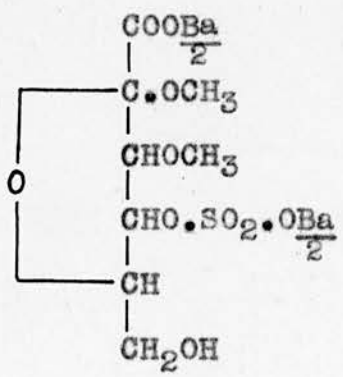
1. Tetramethyl methylgalactoside was removed from a small portion of the distillate by conversion to the crystalline anilide (page 67), and the residual material was shown to be a barium salt, $[\alpha]_D^{16^\circ}$ + 18.3° in water. This method has been used by Smith (5) to separate galactose and arabinose derivatives.

2. It was found that N sulphuric acid at room temperature/

temperature hydrolysed, at least to some extent, the non-galactosic portion of the distillate. Neutralisation of the acid solution with barium carbonate, and removal of the tetramethyl methylgalactoside with chloroform, again yielded a methylated barium salt, $[\alpha]_D^{16^\circ}$ + 21.1° in water (page 68). This was reducing, and gave iodoform on treatment with alkaline hypoiodite.

Further investigation of these salts was prevented by lack of material.

Hydrolysis of methylated H.E. with 4% methanolic hydrogen chloride in nitrogen (page 69) gave a syrup, which was shown to contain an ester. Treatment of this syrup with barium hydroxide gave an ether-insoluble fraction A, which was found to be a non-reducing barium salt containing sulphate, $[\alpha]_D^{16^\circ}$ +15.8° in water. The analysis figures (OMe, 12.1; Ba, 29.8; SO_4, 23.0%) were in fairly good agreement with a barium dimethyl ketohexonate sulphate:-



Calc. for $C_8H_{12}O_{10}SBa$: OMe, 14.2; Ba, 31.5; SO_4, 22.0%.

On heating with hydrochloric acid, a glass was obtained/

obtained, which was reducing, gave the tests for a ketose, and gave the iodoform reaction. It still contained barium and sulphate, and was neutral to litmus. The ether-soluble syrup from which fraction A had been removed was again treated with barium hydroxide to yield a benzene-insoluble fraction B, which was also shown to be a barium carbohydrate sulphate, but with a higher methoxyl and lower barium content than fraction A, $[\alpha]_D^{15^\circ}$ + 17.2° in water. (Found: OMe, 19.5; Ba, 14.5; SO_4, 21.1%).

The benzene-soluble syrup (fraction C) was then maintained at 145°/0.05mm. in order to distil over most of the dimethyl methylgalactoside (fraction D) and leave behind the remaining non-galactose portion as residue (fraction E). Fraction E was then chromatographed, using the method of Jones (6) for separating methylated methylglycosides, and 3 fractions were isolated (page 74):-

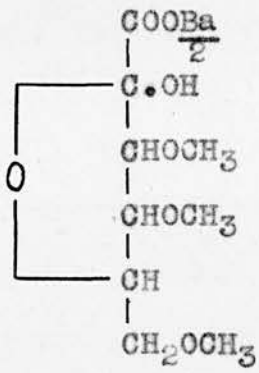
Fraction 1; $n_D^{13^\circ}$ 1.4778.

Fraction 2; $n_D^{13^\circ}$ 1.4738; OMe, 40.0%.

Fraction 3; $n_D^{13^\circ}$ 1.4830; OMe, 33.7%; $[\alpha]_D^{17^\circ}$ + 39.0° in chloroform.

Fraction 1 was not examined, while fraction 2 corresponded to dimethyl methylgalactoside. (Calc. for $C_9H_{18}O_6$: OMe, 41.9%). Fraction 3 on complete methylation and distillation gave a mobile syrup/

syrup (OMe, 52.5; calc. for a fully methylated keto-hexonic acid: OMe, 58.7%). Hydrolysis of this syrup with 0.1 N sulphuric acid at 100° and neutralisation gave a reducing barium salt, the methoxyl content (31.2%) of which agreed with a barium trimethyl ketohexonate:-



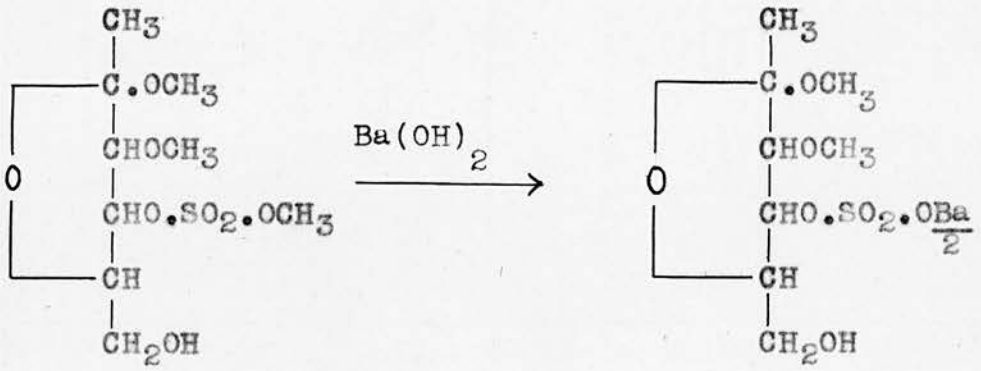
Calc. for $C_9H_{15}O_7\frac{Ba}{2}$: OMe, 30.6%.

The ester was regenerated from the salt by treating with methanolic hydrogen chloride, and converted to the amide, which, however, failed to crystallise after nucleation with tetramethyl fructofururonamide. This is the amide which Young and Rice (10) claimed to have isolated from the methylated H.E. of Chondrus crispus (page 17).

From the above experiments some slight claim may be advanced for the presence of a ketohexonic acid in methylated H.E., but the evidence is far from convincing. For example, in the hydrolysis with 4% methanolic hydrogen chloride (page 69), it seems remarkable that fraction 3 (page 74) should have twice resisted hydrolysis with barium hydroxide under conditions which/

which would decompose an ordinary sugar acid ester rapidly. Again, in the same experiment, fraction A on hydrolysis with hydrochloric acid (page 72) yielded a product giving the iodoform test, which suggests the non-galactosic residue has a $CH_3.CO-$ group in the molecule, unless it be assumed that laevulic acid or some similar product was formed during the acid hydrolysis. Furthermore it is difficult to see how an ester grouping could exist in the original polysaccharide and not be decomposed during methylation in the presence of concentrated alkali.

Certain of the results might also be explained on the assumption that the esters which are present (page 70) are methylsulphuric esters formed by the action of the methanolic hydrogen chloride on the carbohydrate sulphate. This would explain the sluggish hydrolysis with barium hydroxide mentioned above and the fact that a product containing no barium but containing sulphate residues was obtained (page 71). Suppose, for example, that the ester produced on hydrolysis with methanolic hydrogen chloride (page 69) had this structure (OMe, 32.5%):-

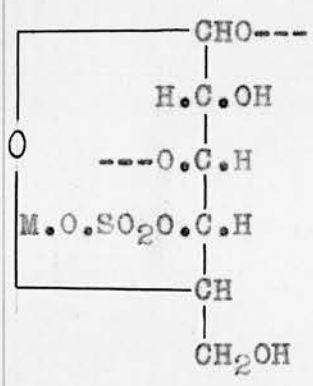


Treatment with barium hydroxide would give rise to a barium salt having Ba, 20.2; OMe, 18.3; SO_4, 28.3%. Fraction B (page 72) had Ba, 14.5; OMe, 19.5; SO_4, 21.1%, and might therefore be a substance of the above type mixed with some sulphate-free material.

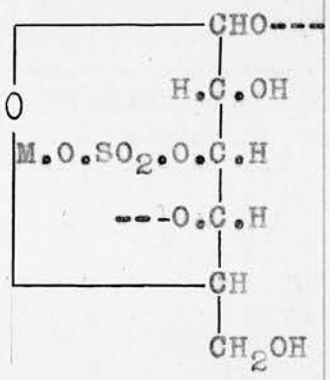
It must be admitted, however, that the high barium content of fraction A (page 71) cannot be explained on the basis that it is exclusively a barium salt of a sulphuric ester, and any proposed constitution of the non-galactosic portion of the polysaccharide molecule must also explain the extreme sensitivity of this residue to acid reagents.

It is only possible at present, therefore, to draw conclusions about the galactose portion of the polysaccharide. From the non-reducing character of the H.E. it is clear that the galactose units are linked through C_1, and the isolation of 2:6-dimethyl galactose excludes both a 1:2- and a 1:6- linkage between adjacent units. The rate of hydrolysis with acid suggests the presence of a pyranose ring, which excludes the possibility of a 1:5-/

1:5-linkage. Hence the linkage must be either 1:3- or 1:4-. On the assumption that the sulphate group is attached to galactose in H.E., as in the Chondrus polysaccharides, (9), the two possibilities are:-



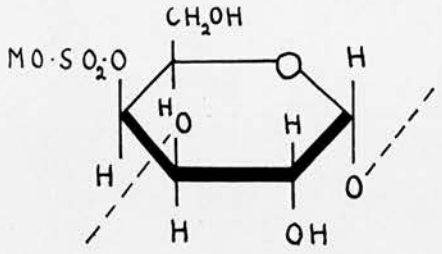
I.



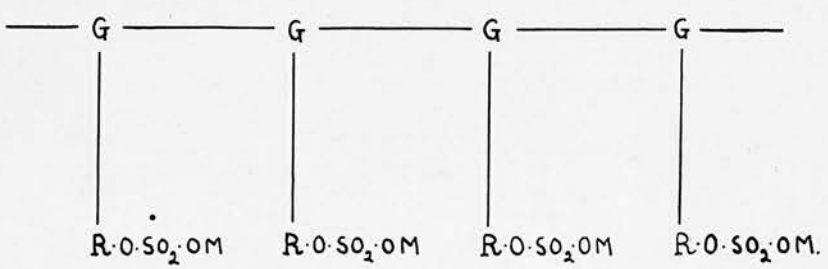
II.

$$M = \frac{Ca}{2} \text{ or } \frac{Mg}{2}$$

II would be readily converted by aqueous caustic soda into a 3:6-anhydrogalactose unit, while I would be expected to resist hydrolysis because of its inability to form an anhydro-ring. It has been shown in Part I (page 34) that the sulphate group is resistant to hydrolysis, thereby indicating that the galactopyranose units are linked to adjacent units through C_1 and C_3, with the sulphate group on C_4. From the positive rotations of H.E. and its acetylated and methylated derivatives, and from the isolation of d-galactose on hydrolysis, the linkage is assumed to be α-1:3-, as in the Chondrus polysaccharides:-



The objection to the above hypothesis is that there is no direct evidence that the sulphate residue is attached to galactose. Indeed, the identification of fractions A and B, from the 4% methanolic hydrogen chloride hydrolysis, as barium carbohydrate sulphates (pages 71-72) rather suggests that the sulphate group may be attached to the non-galactose residue. It is possible to conceive a structure in which the galactose units are linked directly by α-1:3-linkages, with the non-galactose residues, containing the sulphate, attached to C_4 of the galactose unit:-



G = galactose unit.

R = non-galactose unit.

This structure, however, would demand the presence of G and R in equimolecular amounts, while the proportion of galactose to the galactose-free syrup has been shown in Part I to be greater than 1 to 1 by weight. Moreover, the extremely poor yields of non-galactose residue isolated from methylated H.E., together with the failure to identify its structure, make speculation along these lines dangerous, and much further work will be necessary before the precise constitution of the polysaccharide can be laid down.

SUMMARY.

1. The H.E. on acetylation followed by methylation yielded a product which had OMe, 18-20%.

2. The methylated H.E. was still a calcium-magnesium salt of a polysaccharide ethereal sulphate.

3. Complete hydrolysis of methylated H.E., followed by glycoside formation, gave dimethyl methylgalactoside (37%), but no trace of the non-galactose portion of the molecule was obtained.

4. The dimethyl methylgalactoside yielded crystalline 2:6-dimethyl β-d-galactopyranose. This sugar has been characterised by the preparation of the acid, lactone, amide, phenylhydrazide, β-methyl-galactoside, 3:4-monoacetone β-methylgalactoside, and anilide. The properties of these compounds are in good agreement with those recorded by Dr. Bell for the synthetic products.

5. Attempts to identify the non-galactose residue have, for the most part, been unsuccessful. Hydrolysis with 4% methanolic hydrogen chloride in nitrogen gave two barium carbohydrate sulphates in small yield, which had some of the properties of a partially methylated ketohexonic acid. Some evidence for a ketohexonic acid was also obtained from the fully methylated hydrolysis mixture.

6. From the isolation of 2:6-dimethyl galactose the chief building unit of the polysaccharide is assumed to be an anhydrogalactopyranose 4-sulphate, which is joined to adjacent units by an α-1:3-linkage as suggested for the polysaccharides from Chondrus crispus. Other possibilities may be envisaged, however.

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